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EXPERIMENTS ON THE CONTROL OF COTTON ROOT ROT IN ARIZONA [1]

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INTRODUCTION

In recent years several measures have been proposed for the control of cotton root rot, [2] a plant disease which perhaps ranks first in economic importance in Texas, Arizona, and New Mexico. These measures when put into practice by farmers in Arizona have almost invariably proved disappointing. Besides causing a direct annual loss in Texas which some investigators (*8*) [3] estimate at from 15 to 30 millions of dollars, and in Arizona from 1 to 5 millions, the disease is also responsible for considerable depreciation of land values by rendering thousands of acres unfit for the kind of crops which ordinarily produce the greatest profits.

REVIEW OF CONTROL EXPERIMENTS IN TEXAS

Shear and Miles (*6*), from the results of experiments conducted in Texas in 1906 and 1907, recommended deep fall plowing combined with rotation of crops, using crops of grasses or grains for two or three years previous to planting cotton. The practice of fall plowing is not adapted to the Salt River Valley, Ariz., where climatic conditions favor the continued development of the cotton plants until late in the fall, harvesting usually not being completed until January. Experiments with deep spring plowing have shown little or no benefit.

The senior writer has pointed out in former publications (*2*, *3*) the possibility of exterminating the disease in small isolated areas by the use of formaldehyde solutions, but this method is obviously not applicable for general conditions, owing to the expense and labor involved.

The drastic method of clean cultivation, combined with fallowing and crop rotation, recently recommended by Taubenhaus and Killough (*8*), of the Texas station, encounters serious difficulties when applied under Arizona conditions. The cotton plants in this region have a tendency toward perennial habits, and experiments have shown that the roots often penetrate to a depth of 8 or 10 feet and remain partially preserved for many months in spite of thorough plowing and cultivation (pl. 1, A). Therefore the problem of exterminating all the live root tissue by the usual methods of culture becomes very complicated. The high land values in the principal

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[2] Root rot is caused by a fungus, *Phymatotrichum omnivorum* (Shear) Duggar (*1*), which lives in the soil and attacks the roots of cotton and other dicotyledonous plants, causing the plants to wilt suddenly and finally die. Its occurrence in cotton is confined to the Southwestern States, chiefly Texas, Arkansas, Oklahoma, and Arizona.

[3] Reference is made by number (italic) to "Literature cited," p. 309.

A.—Taproots of cotton plants, cut off by the plow in the fall about 8 inches below the surface of the ground, still alive April 1. The longest root reaches a depth of about 8 feet.

B.—Showing preparation of furrows in which manure and other organic materials were buried before planting cotton in the effort to control the cotton root-rot disease.

cotton-growing areas of Arizona, with taxes, water assessments, etc., totaling approximately $20 per acre each year, preclude any general practice of fallowing, and rarely can profitable farming be maintained over a period of several years with a system of successive grass or grain crops.

That the program for exterminating the root-rot organism by eradicating all susceptible plants and their roots can not be undertaken lightly was demonstrated by an experiment at the United States field station, Sacaton, Ariz., in 1921. In January, 1920, an area of approximately 1 acre, which had not been cropped for several years and which had become overgrown with Bermuda grass, was inclosed and pastured to sheep until late in the following fall. In selecting a location for a cotton breeding block for 1921, it was supposed that this pasture area on which all weed growth had been held in check by the sheep, would be free of root rot. The preliminary preparations were made late in the fall so as to gain early control of the Bermuda grass. After thorough plowing and disking, all roots and other live plant material that could be collected by the use of harrows, sulky hay rake, and hand rakes were gathered and burned. Repeated disking prevented the growth of grass or weeds during the winter, and after planting the cotton the most painstaking efforts were made with hand and horse implements to prevent the renewal of grass or weed growth. These drastic efforts succeeded in controlling the Bermuda grass, at which they were largely aimed, but since much of the cotton was killed by an attack of root rot which began in midsummer, it was obvious that these precautions were not sufficient for controlling the root-rot disease.

In reviewing the literature on the subject of control of root rot in Texas, it is noted that nearly all of the investigators have studied the effect of applications of manure, and for the most part the results reported have been negative. Youngblood (*9*) credits Shear and Miles with having found that applications of farmyard manure in a large measure controlled the disease, but the writers have been unable to locate the source of this information in the publications by Shear and Miles (*6*, *7*). Scofield (*5*) has found, from rotation experiments at the San Antonio Experiment Farm, Tex., covering a period of 7 years, "that the use of farmyard manure has not materially reduced the injury from root rot." Taubenhaus and Killough (*8*), after 5 years of experiments in Texas, state that "it is doubtful if the addition of humus or manure to the soil will greatly influence root-rot control, although it may increase the yield in cotton."

EXPERIMENTS WITH MANURE IN THE SALT RIVER VALLEY, ARIZONA

After having determined by field survey the inadequacy of deep plowing and continuous planting to grain crops as a control measure in the Salt River Valley, the senior writer undertook some investigations in 1917 to determine the effectiveness of applications of manure. A circular spot of 0.7 acre, on which the percentage of dead cotton plants had been determined the previous fall, was selected in March, 1918, and on one half of the area, representing 26 short rows, 10 tons of dry farmyard manure were applied, the other half of the area being left as a control. The entire area was plowed a few days after

the application and planted to Pima Egyptian cotton. Counts were made of the percentage of plants killed by root rot at different intervals during the season. The data obtained are shown in Table I.

TABLE I.—*Percentage of Pima cotton plants killed by root rot during different periods of the growing season and the yield of seed cotton on manured and untreated infected areas near Phoenix, Ariz., in 1917–18*

	Percentage of plants dead on Nov. 15, 1917	Percentage of plants dead, 1918			Yield of seed cotton	Yield on acre basis
		July 15	Aug. 13	Oct. 18		
					Pounds	*Pounds*
Manured	90	0	3	59	445	1,271
Untreated	91	10	30	86	100	286

In addition to the difference in the final count of dead plants, there is evidence that the incidence of the disease was delayed on the treated areas, which gave the majority of the plants the opportunity to produce almost a full crop. Subsequent studies have confirmed this effect of manure on the disease.

In 1919 a similar experiment was conducted by the senior writer in cooperation with a large cotton plantation near Chandler, Ariz., where approximately 280 of the 8,000 acres which were cleared from the desert in 1917 and 1918 were found to be severely spotted with root rot the first year of cultivation. In this experiment an area of approximately 0.45 acre was treated with manure applied at the rate of 16 tons per acre about four months before planting the cotton, and an equal area to which no manure was applied was used as a control. The percentage of dead plants and the yield of seed cotton are given in Table II.

TABLE II.—*Percentage of Pima cotton plants killed by root rot, and yield of seed cotton, on manured and untreated infected areas near Chandler, Ariz., in 1918–19*

	Percentage of plants dead November, 1918	Percentage of plants dead November, 1919	Yield of seed cotton	Yield of seed cotton per acre
			Pounds	*Pounds*
Manured	67.7	35.6	493	1,096
Untreated	61.4	64.2	404	898

In view of the well-known erratic behavior of the disease from year to year (*4*), it is unfortunate that these experiments were not run longer than one year. However, changes in plans, due to the transfer of the plantation management to other hands, and the discouraging reports of manure applications in Texas experiments, caused these studies to be discontinued until 1921, when they were resumed in collaboration with the junior writer at the United States field station at Sacaton, Ariz.

EXPERIMENT WITH ORGANIC MATERIALS AT SACATON, ARIZ.

In December, 1920, a plot of one-fourth acre in the center of a 2½-acre area which had shown severe infection each year since 1907, was plowed immediately after the cotton was harvested. It was then furrowed out with a lister and by hand shoveling until seven deep wide furrows or ditches were prepared the same distance apart as the cotton rows (pl. 1, B). Four of these were filled with manure at the rate of 12 tons per acre and the other three with green alfalfa at the rate of 15 tons per acre, covered by disking, leveled, and allowed to remain until planting time in the spring. Pima Egyptian cotton seed was then planted directly over the organic material. This same procedure was followed on the same plot (C2–19) preliminary to planting in 1922, 1923, 1924, and 1925, except that manure was used in all furrows.

Beginning in 1923 the experiment was extended to include the entire infected block, alternate plats of seven rows each being treated with organic materials and the others being used as controls. Plats C2–17, 19, and 21 were given applications of manure, while plat C2–23 was treated with spoiled alfalfa hay at the rate of 8 tons per acre.

Beginning in the fall of 1919, the infected areas in the 2½-acre plot were charted, as indicated by the dead cotton plants, at the end of each crop season. The percentage of area infected, as determined by these measurements in all of the plats from 1919 to 1925, inclusive, is shown in Table III, and Figures 1 to 6.[4]

TABLE III.—*Percentage of area infected with root rot in plats of Pima cotton, manured and unmanured, at the United States Field Station, Sacaton, Ariz., 1919 to 1925, inclusive*[a]

	1919	1920	1921	1922	1923	1924	1925
C2–24. Control				14.5	38.4	70.9	62.6
C2–23. Manured, 1923–25	43.0	63.0	55.4	51.6	*9.7*	*10.2*	*6.1*
C2–22. Control	68.9	65.3	30.9	60.8	29.7	22.4	18.9
C2–21. Manured, 1923–25	60.4	70.4	37.9	56.6	*14.6*	*14.4*	*7.5*
C2–20. Control	49.5	47.3	14.6	27.1	15.0	11.6	18.9
C2–19. Manured, 1921–1925	57.1	71.7	*13.8*	*14.1*	*3.7*	*2.2*	*1.1*
C2–18. Control		77.3	46.4	65.3	24.1	22.9	24.1
C2–17. Manured, 1923–25	51.3	61.9	51.8	65.6	*11.8*	7.4	*5.3*
C2–16. Control		39.4	36.8	53.8	9.2	16.2	26.1

[a] The figures for 1925 became available during publication and afford further evidence of the beneficial effects of the application of manure in reducing root-rot injury.

Italicized percentages indicate that manure was applied to the plat prior to planting.

As may be seen from Table III and Figure 5, there was an abrupt reduction of area infected by root rot on all of the plats immediately following the first application of manure. There was a diminution of infected area in all the plats in 1921 as well as on plat C2–19, but the reduction on the untreated plats was not nearly so great, nor was it maintained in the 3 years following as on the treated plat (figs. 3, 4, 5, and 6). The data show also that less area was infected in all of the plats in 1923 and 1924 than in 1922, except plat C2–24 on which there had been no cotton planted for many years before 1922.

[4] The 1919 field chart, fig. 1, was prepared from data obtained by S. H. Hastings, who was superintendent of the station at that time. The later data, figs. 2 to 6, were obtained by the writers.

This general reduction necessarily must be attributed to the seasonal conditions of those years, since the cultural conditions were much the same as in previous years. The reduced area of infection in the treated plats following the manure applications was either main-

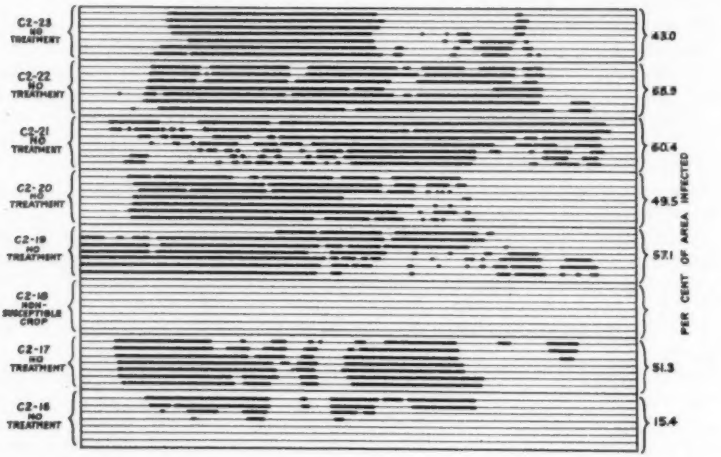


FIG. 1.—Diagram showing areas infected by root rot in experimental plats of Pima cotton at Sacaton, Ariz., at end of season 1919, prior to treatment with organic material. Heavy lines indicate infected areas

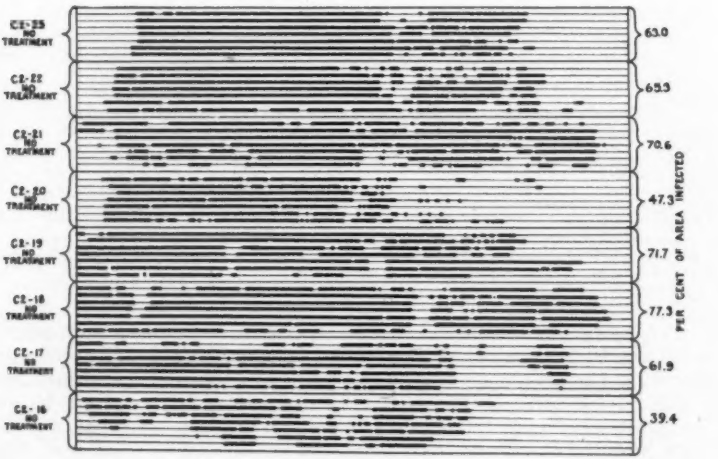


FIG. 2.—Diagram showing areas infected by root rot in experimental plats of Pima cotton, at Sacaton, Ariz., at end of season 1920, prior to treatment with organic material. Heavy lines indicate infected areas

tained within 1 per cent of the year previous or was further reduced the succeeding year, whereas considerable fluctuation occurred in the area infected in control plats.

FIG. 3.—Diagram showing areas infected by root rot in experimental plats of Pima cotton, at Sacaton, Ariz., at end of season 1921. Plat C2-19 received an application of organic material prior to planting. Heavy lines indicate infected areas

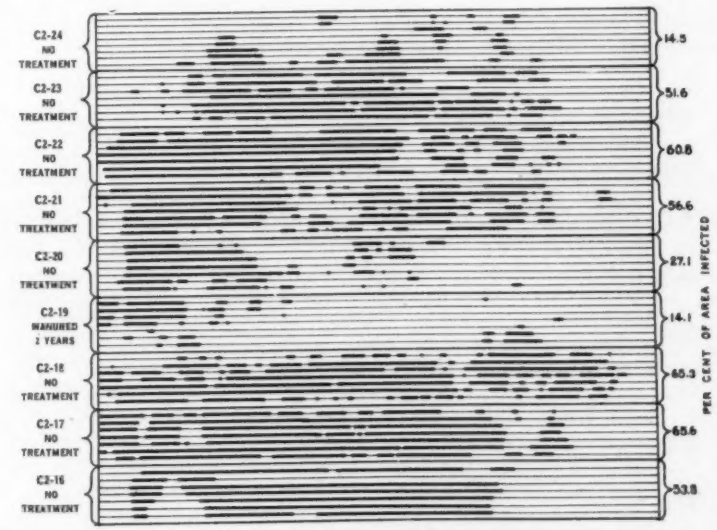


FIG. 4.—Diagram showing areas infected by root rot in experimental plats of Pima cotton, at Sacaton, Ariz., at end of season 1922. Plat C2-19 had received applications of organic material prior to planting in 1921 and 1922, while the other plats had received no fertilizing treatment. Heavy lines indicate infected areas.

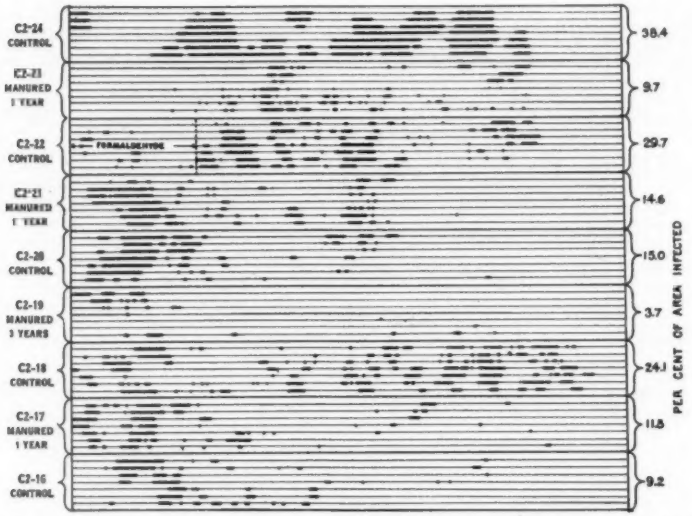


FIG. 5.—Diagram showing areas infected by root rot in experimental plats of Pima cotton, at Sacaton, Ariz., at end of season 1923. Plat C2-19 had received applications of organic material for three years; and plats C2-17, C2-21, and C2-23 received applications for one year. The area treated with formaldehyde in plat C2-22 was not included in figuring the per cent of infection in this plat. The formaldehyde was applied immediately before planting and the infection at the end of the season was 6.9 per cent in contrast to 88.2 per cent the previous year. Heavy lines indicate infected areas

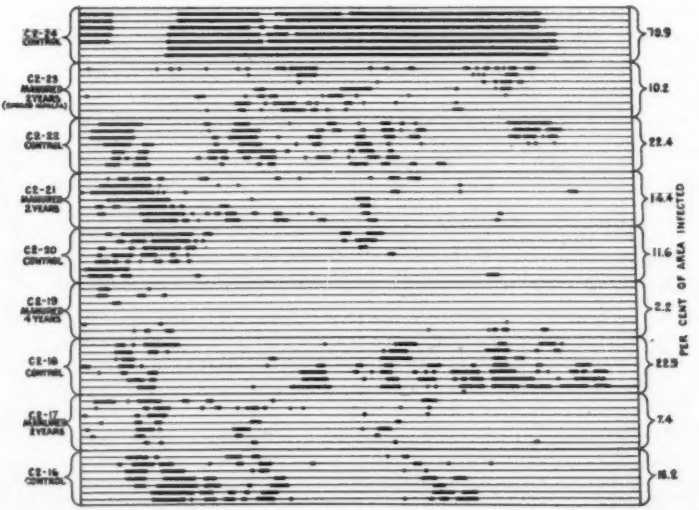


FIG. 6.—Diagram showing areas infected by root rot in experimental plats of Pima cotton, at Sacaton, Ariz., at end of season 1924. Plat C2-19 had received applications of organic material for four years. Plats C2-17, C2-21, and C2-23 had received applications for two years. Heavy lines indicate infected areas

The percentage of plants killed by root rot in each row, as determined by actual count in 1923 and 1924 on plat C2–19 and on control plats C2–18 and C2–20 which were on either side of it, is shown in Table IV.

TABLE IV.—*Percentage of cotton plants per row killed by root rot in plat treated with manure for four years and in two adjacent untreated plats* [a] *at the United States Field Station, Sacaton, Ariz., 1923 and 1924* [b]

Row No.	C2–18 (control)		C2–19 (manured 4 years)			C2–20 (control)		
	Percentage of plants killed		Row No.	Percentage of plants killed		Row No.	Percentage of plants killed	
	1923	1924		1923	1924		1923	1924
1	26.6	34.3	1	6.6	5.4	1	8.4	5.2
2	31.4	27.1	2	1.4	0.3	2	8.8	6.3
3	30.8	23.7	3	0.0	0.0	3	12.2	4.6
4	25.1	16.3	4	0.3	0.0	4	16.1	7.4
5	23.1	12.8	5	2.0	1.4	5	12.9	10.0
6	22.3	14.0	6	4.8	2.8	6	15.7	15.1
7	24.9	18.5	7	6.3	1.7	7	20.4	22.5
Average per cent	26.6	20.9		3.5	1.6		13.5	10.2

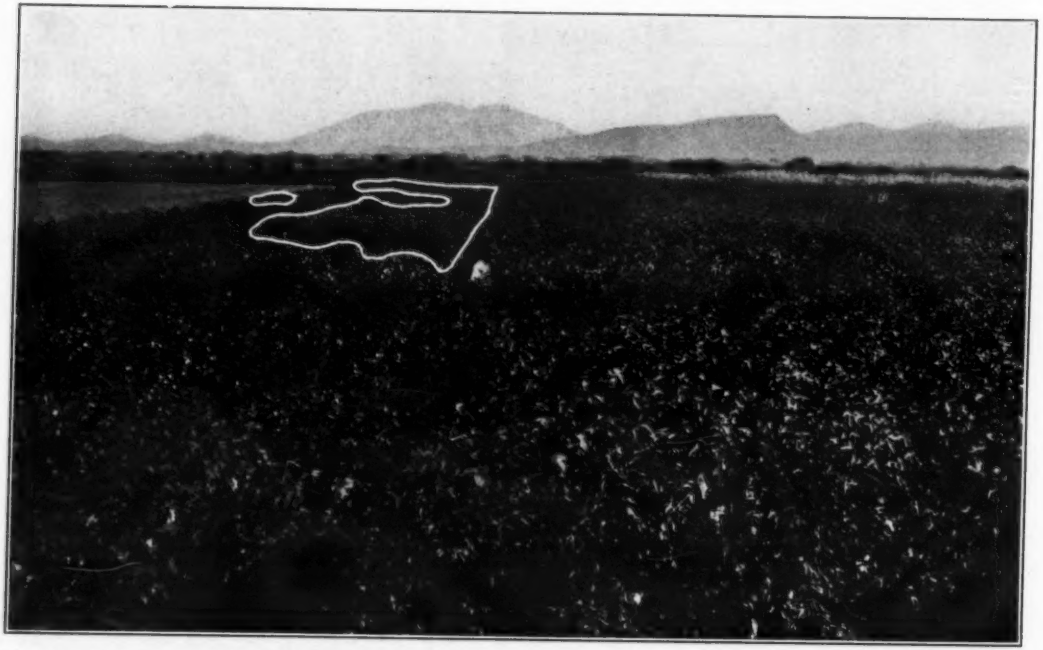
[a] The apparent discrepancy in the degree of infection of plats C2–18, C2–19, and C2–20, shown in Tables II and IV, is due to the fact that the data in Table III represent measurements of infected soil area while hose in Table IV represent the number of dead plants by actual count.

[b] The count of dead plants was made Oct. 30, in 1923, and Oct. 2, in 1924.

It will be noted that the difference in the amount of infection between the plat manured for four years and the adjacent control plats is maintained consistently in each row, although rows 1 and 7 on plat C2–19 are separated by only 3½ feet from outside rows of the control plats. Every plant in row 3 of plat C2–19 reached maturity and produced a full crop without interference from the disease in both 1923 and 1924. Row 4 showed no infection in 1924; and row 2 was practically free from infection during both years. Prior to 1923 there had never been observed one entire row free from infection in the entire 2½-acre area. From Table IV and Figures 5 and 6 it will be noted that nearly all of the infection in plat C2–19 occurred in the outside rows 1 and 7 which were contiguous to the control plats, and on rows 2 and 6 which were the next most exposed to infection from without. It was obvious during the disease activity that a greater part of the infection in these outside rows actually occurred from invading mycelium from the control plats.

The marked reduction in the extent of infection following the manure treatment (figs. 3, 4, 5, and 6) can scarcely be explained by the migratory habits of the disease.

The earliest chart, that for 1919 (fig. 1), shows that the infection in plat C2–20 did not extend as far to the north as in the 6 neighboring plats, and after the season 1920 a gradual diminution or "clearing up" of the infection on the north end is observable, which can not be attributed to any cultural treatment applied. There is a possibility that this same unknown factor may have operated in the



Showing differences in extent of root rot infected areas in plats of Pima cotton which had received an application of spoiled alfalfa and which had not. The alfalfa was applied to the land on the right, and not applied to that on the left

reduction of the infection on the north ends of the contiguous manured plats C2–19 and C2–21. It becomes difficult, however, to explain by such reasoning the almost complete disappearance of the infection from plat C2–19 with considerable infected area on either side of it. Likewise, the occurrence of the badly infected area on the north end of control plat C2–18, with little or no infection adjacent in 1923 and 1924, can hardly be attributed to the erratic habits of the disease. Moreover the striking contrast in the extent of area infected in plats C2–23 and C2–24, separated by only a slight ridge 2½ feet wide, seems to indicate clearly that a cultural factor is responsible (pl. 2).

The full importance of the degree of control indicated by the applications of organic material is not expressed in the tables and charts given above. It appeared that an additional benefit was derived from the use of organic materials, in that the disease activity was delayed until later in the season, which allowed the plants time to produce nearly a full crop. An indication of this occurrence may be seen in the record of dead plants counted at different intervals in 1918 and 1924 (Tables I and V).

TABLE V.—*Number of cotton plants killed by the root-rot disease during intervals of two weeks on one-fourth acre manured and control plats at the United States Field Station, Sacaton, Ariz., in 1924* [a]

	July 31	Aug. 15	Sept. 2	Sept. 17	Oct. 2	Total dead plants
C2-23. Manured 2 years, spoiled alfalfa..	5	17	54	78	51	205
C2-22. Control	38	76	186	163	96	559
C2-21. Manured 2 years	3	17	101	114	97	332
C2-20. Control	18	13	58	93	67	249
C2-19. Manured 4 years	4	4	14	15	4	41
C2-18. Control	22	102	136	141	113	514
C2-17. Manured 2 years	4	22	31	28	28	113
C2-16. Control	21	42	116	129	109	417

[a] The number of plants per plat was approximately 2,600.

In Table V it will be observed that little disease activity occurred in the manure-treated plats until some time after August 15, while in the control areas much damage had already been done by that date. Since it is usual for some bolls to be fully mature in late August in Arizona, any deterrent to the activity of the disease prior to this date would be highly advantageous.

EFFECT OF MANURING ON THE ROOT DEVELOPMENT OF COTTON PLANTS

In an effort to determine the cause of the apparent influence of manuring on the root-rot disease, the writers, on December 5, 1924, exhumed for study a number of cotton root systems from uninfected rows 3 and 4 of plat C2–19, and also from apparently uninfected areas about 30 feet distant in the adjacent control plats C2–18 and C2–20. In selecting groups of plants in the three plats close together, it was thought that differences in root development due to soil differences would be largely avoided. The plants exhumed from the manured plat were about 15 feet distant from the nearest dead plants,

but it was impossible, at this time, to find a group of uninfected plants on the control plats without some dead plants near by.

The root systems in the manured plat were more superficial than those in the control plats, and the lateral roots near the surface of the ground were larger and more numerous. A greater number of small, fibrous roots were also found in the top soil in the manured plat. In the control areas, the taproots were usually found in an almost vertical position, and they extended to a depth of 4 or 5 feet in this position; whereas in the manured area the taproots frequently divided into several laterals at a depth of 10 to 20 inches and failed to penetrate the deeper layers of soil.

The percentage of these aborted (divided) or deflected taproots, and the mean number of lateral roots per plant in the top 10 inches of soil in the different plats, is shown in Table VI.

TABLE VI.—*Percentage of aborted or deflected taproots per plant in the top soil of a plat manured for four years and of two control plats, in 1924*

Plat No.	Number of plants examined	Percentage of aborted or deflected taproots	Mean number of lateral roots in top 10 inches of soil
C2–18. Control	9	22.3	12.0
C2–19. Manured four years	13	46.2	15.3
C2–20. Control	8	12.5	12.6

Although the plants in manured plat C2–19, whose roots were examined, were apparently healthy at the end of the season and were several feet distant from any plants which had died from root rot, the roots of 6 of the 13 plants studied were partially diseased. In some cases only the tip of one or two lateral roots was destroyed, but in others two or three laterals were entirely rotted and lesions were found on the taproots. Diseased roots were found in control plat C2–18 also, but this was not unexpected, as dead plants were located only 3 or 4 feet away. No infected roots, however, were found on the plants studied on control plat C2–20, although plants which had died from root rot were only 6 feet distant. Such an occurrence suggests that the application of organic matter in the manner described may not be materially effective in exterminating the root-rot fungus, but that it may so modify the soil as to affect the physiology of the plants in such a way as to enable them to partially or entirely withstand the attack of the organism.

Instances of deciduous trees remaining alive for many months after part of the root systems had been destroyed by root rot have been observed by the writers many times. It is not improbable that the development of a more extensive and a more superficial root system than normal for the cotton plant might give better defense against the disease, and the possibility of perfecting control measures along such lines is suggested.

SUMMARY

Root rot is the most serious disease affecting cotton, alfalfa, fruit trees, and many other economic plants in the valleys of southern Arizona.

It has been found impracticable to apply in Arizona the control measures recommended for Texas.

The results of various experiments reported from Texas, in which applications of manure and other organic materials were used as control measures, indicate that the disease injury was not reduced by the treatment.

Experiments conducted in the Salt River Valley and at Sacaton, Ariz., to test the effects of manure and other organic materials on the control of root rot, have consistently shown a reduction in the infected area and the number of cotton plants dying from the disease following the treatment.

In a plat which for several years was the most seriously infected of a group of 9 quarter-acre plats, the infected area was reduced from 71.7 per cent in 1920 to 2.2 per cent in 1924, following 4 years of manuring treatment which was started in 1921. Of the 7 rows of cotton, 410 feet long, in this manured plat, the 3 inner rows were practically free from infection in 1923, and 2 of them in 1924 were entirely free from disease injury.

By recording the progressive damage of the disease at different intervals during its activity it was found that the incidence of the disease was delayed in the manured areas, which allowed some of the plants time to produce nearly a full crop.

An examination of the root systems of cotton plants growing in the plat treated with organic materials for four years, and in the adjacent untreated plats, showed the former to be more superficial, with larger laterals and more fibrous roots near the surface of the ground.

Several of the plants in the manured plats, although healthy in appearance above ground, were found to have infected and partially decayed roots. This suggested the idea that the fungus may be little affected by applications of organic materials, but that the plants may be better equipped to avoid or withstand the disease under the modified soil conditions.

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ROOT ROT OF THE BEAN IN CALIFORNIA CAUSED BY FUSARIUM MARTII PHASEOLI BURK. AND F. ADUNCISPORUM N. SP.[1]

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INTRODUCTION

Root rot of the bean is a widespread disease in California, where it occurs in all of the bean-growing sections and on all varieties. The percentage of plants affected varies considerably, not only in different fields but also in different parts of the same field. Some entire fields and large areas in others have been seen where practically 100 per cent of the plants were affected, and in such cases there is great financial loss to the grower.

SYMPTOMS

In the early stages the affected plants usually show no aboveground symptoms of root rot. Sometimes, however, symptoms of the disease appear very early on the seedlings, which suggests that they may possibly have been diseased from the start. A few weeks after the plants have come up, individual plants or groups of plants are stunted and more or less yellowed. Such plants may eventually die. Usually, however, the plants die very slowly, a majority of them living throughout the growing season but remaining stunted and producing few or no seed.

Although only one or two distinct reddish lesions may be present on the taproot, it is more common to find a more or less uniform reddish discoloration of a major portion of the taproot, sometimes extending slightly above the surface of the ground and to the larger fibrous roots. At first little decay is evident. However, the disease gradually progresses inward and upward, and the fibrous roots and the lower part of the taproot shrivel and finally become dry. Frequently small lesions are found on the taproot at the base of small decayed lateral rootlets, indicating that the causal organism had entered at that point. However, in many cases the lesions on the taproot are in no way associated with decaying rootlets, the inference being that infection can take place directly through the epidermis or through wounds. Sometimes a large number of fibrous roots develop just below the surface of the soil.

The symptoms of the root rot of beans in California correspond very closely to those described by Burkholder [2] for the same disease in the State of New York. Other root rots of the bean occur in California, but they will not be discussed in this paper.

[1] Received for publication Aug. 7, 1925; issued February, 1926.

[2] BURKHOLDER, W. H. THE DRY ROOT-ROT OF THE BEAN. N. Y. (Cornell) Agr. Expt. Sta. Mem. 26: 1003–1033, illus. 1919.

ETIOLOGY

While making a survey of bean diseases in California during the summer of 1924, the writers undertook to determine the cause of the root rot described above. Numerous isolations were made from decaying roots of several varieties of beans, including Blackeye cowpeas, growing in most of the big bean-producing sections. Some species of Fusarium was almost invariably obtained. Those species which appeared most commonly as well as many others were studied on different culture media and their parasitism tested. These studies showed that out of 40 different cultures of Fusarium isolated from typical root-rot lesions from as many different plants, only 7 were parasitic under the conditions of the experiments. Four of these proved to be *Fusarium martii phaseoli* Burk.; one *Fusarium tracheiphila* (Erw. Sm.) Wr., isolated from and parasitic on Blackeye cowpea (*Vigna sinensis* Hassk.); and two were the new species named and described below. The other nonparasitic species were mostly to the Elegans type, but a few belonged to the Martiella and Discolor groups.

NAME

The name *Fusarium aduncisporum* n. sp. is proposed for the new species. The term "aduncus," meaning hooked, describes one of the striking morphological characters which distinguishes this species from other closely related forms.[3]

DESCRIPTION

Microconidia not common. Macroconidia, of approximately uniform diameter throughout or tapering slightly towards one or both ends, distinctly curved. One end, rarely both, may be bent inward, becoming more or less hook-shaped; rounded at both ends, not at all or slightly pedicellate, typically 0- to 3-septate, less commonly 4-septate, rarely 5-septate, septations frequently not visible. Macroconidia $48.3 \times 5.3\mu$ (60 to 30×4.5 to 5.5μ). Chlamydospores mostly intercalary, borne singly or in short chains, not common on most media, 5 to 12μ. Aerial mycelium poorly developed, usually white. Spores borne in sporodochia and pseudopionnotes, light-buff to zinc-green in color on potato cylinders, light to warm buff on potato agar, and from light-buff to grayish-olive to Bister on string-bean plugs.[4] Deep vinaceous color imparted to the medium when grown on oatmeal agar and flesh-pink to jasper-pink on rice. On most media the conidia anastomose freely, forming compact masses which can be separated only with difficulty.

In roots of bean (*Phaseolus vulgaris* Linn.), California. This fungus was isolated twice, both times from Kentucky Wonder beans growing at Carlsbad and Ventura, Calif.

Measurements of spores on various culture media are as follows:

On potato cylinders, 11-day-old culture, conidia from Sporodochium: 0-septate,[5] average $45.6 \times 5\mu$, range 27.5 to 59×3.8 to 5.6μ.

[3] The writers are indebted to H. W. Wollenweber and C. D. Sherbakoff for examining cultures of the organism, and to the latter for suggesting the name used.

[4] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

[5] Spores in which no septa were visible were classed as 0-septate. In many cases the septa may have been obscured by the dense protoplasmic contents.

On oat agar, 21-day-old culture, conidia from sporodochium: 0-septate, average 49.7×5.5μ, range 32.5 to 57.5×5 to 5.9μ; 1-septate, average 51.6×5.5μ, range 46 to 58.8×5 to 5.6μ; 2-septate, average 54×5.6μ, range 41 to 56×5 to 5.6μ; 3-septate, average 51.8×5.6μ, range 50 to 54×5.6μ.

On beef agar, 21-day-old culture, conidia from pseudopionnotes: 0-septate, average 44×5μ, range 37.5 to 54×5μ; 1-septate, average 49×5μ, range 46 to 59×5μ; 3-septate, average 48.6×5.2μ, range 41 to 55×5 to 5.6μ; 4-septate, average 50×5.2μ, range 47.5 to 55×5 to 5.6μ.

On corn agar, 22-day-old culture, conidia from sporodochium: 0-septate, average 43.7×5.5μ, range 25 to 52×5 to 5.9μ; 1-septate, average 39×5.5μ, range 35 to 41×5 to 5.9μ; 2-septate, average 47.3×5.5μ, range 45 to 50×5 to 5.6μ; 3-septate, average 38.8×5.6μ.

On lima-bean agar, 22-day-old culture, conidia from sporodochium: 0-septate, average 45×5μ, range 34 to 56×4.4 to 5.6μ; 1-septate, average 46.8×5μ, range 44 to 52.5×5μ.

On string-bean plug, 22-day-old culture, conidia from sporodochium: 0-septate, average 43×5.1μ, range 37.5 to 47×5 to 5.9μ; 2-septate, average 49×5.7μ, range 43.8 to 54×5.6 to 5.9μ.

On stems of *Melilotus alba*, 18-day-old culture, conidia from pseudopionnotes: 0-septate average 52×5.5μ, range 37.5 to 71×3.75 to 5.6μ; 1-septate, average 50×5.6μ, range 39 to 54×5.6μ; 2-septate, average 60×5.4μ, range 56 to 64×4.7 to 5.6μ; 3-septate, average 55.5×5.6μ, range 49 to 62×5.6μ.

Type material has been deposited in the herbarium of the United States Department of Agriculture, Bureau of Plant Industry, Washington, D. C.

Owing to the fact that about 80 to 90 per cent of the ungerminated spores in most cultures show no septations, while a like percentage of the germinated spores show from 1 to 4 septations, it is thought that the septa do not become evident in many spores until germination. On this account estimates of percentages of spores with different numbers of septations are sure to be very inaccurate. Likewise the spores when germinating in culture, as many of them do, become so intertwined that they can scarcely be separated without crushing many of them. A very rough estimate made after an examination of germinated spores from many mounts from several different culture media showed that approximately 1 per cent, 40 per cent, 30 per cent, 20 per cent, and 9 per cent of the spores were 4, 3, 2, 1, and 0 septate, respectively. Now and then a 5-septate spore was seen.

A study of the morphology of this fungus, together with its reaction on various culture media, shows that it belongs to the Martiella group, but that it differs from any hitherto described species in some essential details. The shape and curvature of the spores illustrated in Plate 1 is sufficient to distinguish it at once from any other species of Fusarium of which the writers have knowledge. Its reaction on different culture media shows it to be very closely related to *Fusarium martii phaseoli* Burk. A close physiological relationship is denoted by the fact that it produces exactly the same type of disease in the bean.

Photomicrographs showing different types of the spores of *Fusarium aduncisporum*. ×500

COMPARISON OF FUSARIUM ADUNCISPORUM AND F. MARTII PHASEOLI ON VARIOUS CULTURE MEDIA

Although their morphological differences are sufficient to distinguish *Fusarium aduncisporum* from *F. martii phaseoli*, it was thought desirable to compare them on a number of different culture media. A brief summary of some of the results of these comparisons with 30-day-old cultures is given below:

Steamed rice: Both form coral-pink to flesh-pink color in the medium, and about the same amount of white aerial mycelium. *Fusarium martii phaseoli* has more of a tendency to form purple-drab color on the surface of the medium.

Potato plugs: *Fusarium martii phaseoli* forms zinc-green to dusky green-blue color, while *F. aduncisporum* forms more of a cinnamon-buff to clay color to olive-gray, although it does form some zinc-green and dusky green-blue in some stages of its growth.

String-bean plugs: *Fusarium martii phaseoli* produces patches of a color approaching plum-purple more closely than any color given by Ridgway, while *F. aduncisporum* produces more of a grayish-olive to clay color to Bister.

Melilotus-alba stems: These fungi differ on this medium chiefly in the production of more blue color by *Fusarium martii phaseoli*. This is especially evident in cultures a month old, at which time the sporodochial layers of *F. aduncisporum* are frequently light-buff to nearly white, especially when the excess water has disappeared.

Beef agar: No appreciable difference.

Carrot agar: Cultures are easily distinguished by a large amount of zinc-green to dusky green-blue in *Fusarium martii phaseoli* cultures, as contrasted with olive-buff to nearly white in *F. aduncisporum* cultures.

String-bean agar: *Fusarium aduncisporum* can be distinguished from *F. martii phaseoli* on this medium by the lack of green and blue color and greater amount of cinnamon-buff to clay color.

Oat agar: A slightly deeper pink color is produced in the medium in cultures of *Fusarium martii phaseoli*.

Lima-bean agar: Large amount of zinc-green and dusky green-blue in cultures of *Fusarium martii phaseoli*, and only light-buff in *F. aduncisporum* cultures.

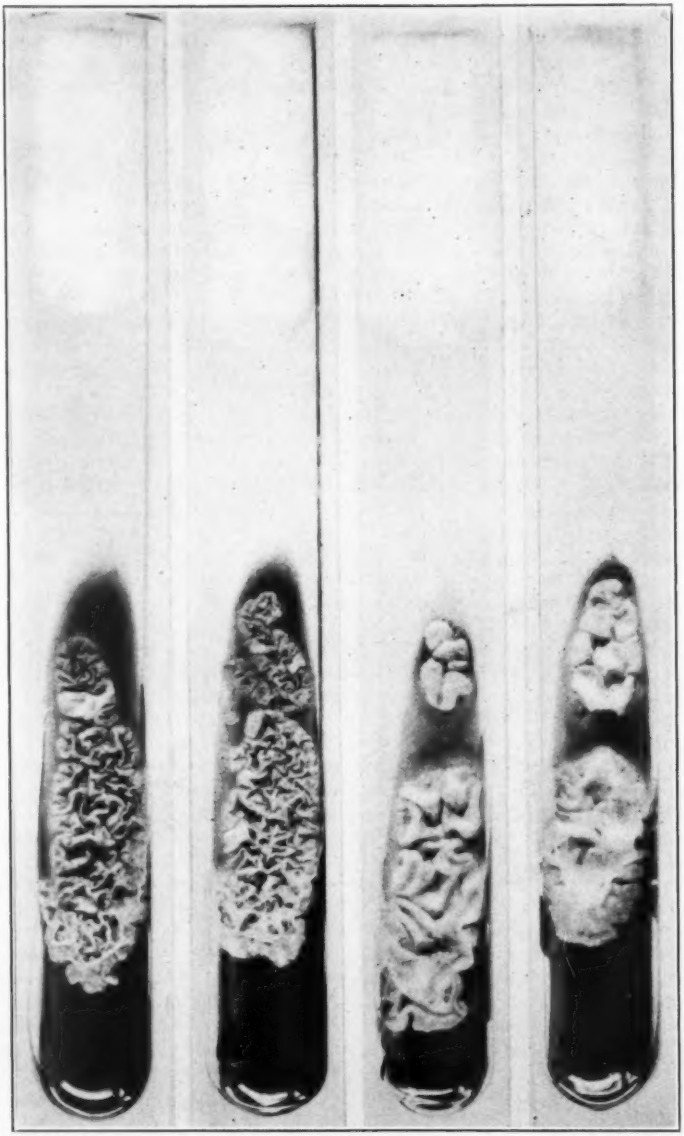
Corn agar: Slightly more blue formed in cultures of *Fusarium martii phaseoli*.

Potato agar: Differ only in presence of zinc-green and dusky green-blue in cultures of *Fusarium martii phaseoli*, as contrasted with light to warm buff in *F. aduncisporum* cultures.

Synthetic agar:[6] Rather striking difference in size of the convolutions in the surface of the membranouslike growth, as illustrated in Plate 2. The convolutions in the *Fusarium aduncisporum* cultures are considerably larger than those in the cultures of *F. martii phaseoli*.

The above comparisons are sufficient to show that although these two fungi can not be distinguished on some media they are quite different on others, the outstanding differences being in the production of more zinc-green and dusky green-blue color by *Fusarium martii phaseoli* and more cinnamon-brown to clay color by *F. aduncisporum*.

[6] Dextrose, 200 gms.; peptone, 10 gms.; ammonium nitrate (NH_4NO_3), 10 gms.; potassium nitrate (KNO_3), 5 gms.; dipotassium phosphate (K_2HPO_4), 2.5 gms.; magnesium sulphate ($MgSO_4$), 2.5 gms.; calcium chloride ($CaCl_2$), 0.10 gms.; agar-agar, 30 gms.; distilled water, 1,000 c. c.



Photographs of cultures of *Fusarium martii phaseoli* (the two on the left, 187) and *Fusarium aduncisporum* (the two on the right, 144A) on synthetic agar, showing the relative size of the convolutions formed by the membranous growth

PATHOGENICITY

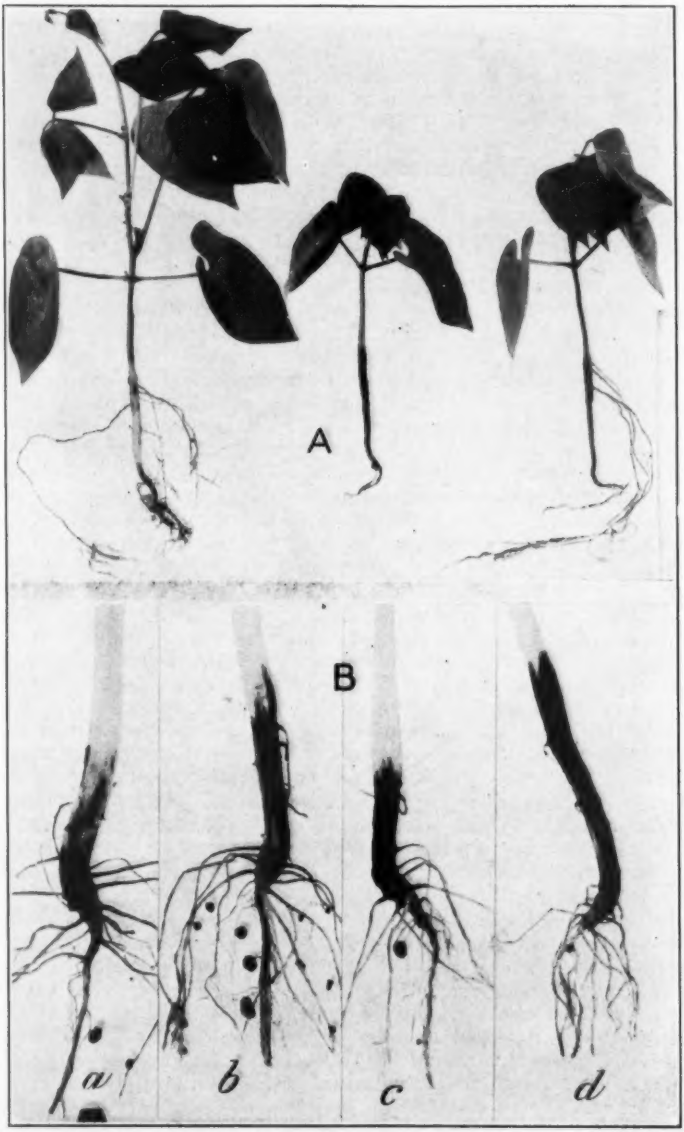
The first evidence that *Fusarium aduncisporum* was parasitic was obtained in an experiment conducted to determine which of the 40 cultures isolated from bean root rot were capable of causing the disease. As a control, a culture of *F. martii phaseoli* obtained from W. H. Burkholder was included. Henderson Bush Lima beans and Blackeye cowpeas were planted in sterilized soil in the greenhouse at the Arlington Experiment Farm at Rosslyn, Va. A spore suspension of the fungus to be tested was poured over the seeds before they were covered. Five pots of Lima beans and 5 of cowpeas—each with 5 seeds, making a total of 10 pots containing 50 seeds—were used for each fungus. Fifteen days after the seeds were planted infection was distinctly evident in the plants in those pots inoculated with *F. martii phaseoli* from Burkholder as well as in those inoculated with cultures of the fungus from California. Likewise infection had developed in two series of pots inoculated with *F. aduncisporum*. The type of root rot produced was the same, and the decay seemed to develop at the same rate in all cases. Practically 100 per cent of the plants of both hosts were affected. The fungi were recovered in pure culture and used for other inoculation experiments with positive results.

To further test the parasitism of these fungi, the pots from which the infected plants were removed were replanted with Henderson Bush Lima seedlings. These were grown in sterilized sand in the greenhouse, and then transplanted soon after they appeared above the surface of the sand. Infection became evident in some plants 11 days after they had been transplanted. Considerable dwarfing was evident in three weeks, at which time the inoculated plants were only about half the size of the controls (pl. 3, A).

After seven weeks, when the experiment was discontinued, 100 per cent of the plants in the pots in which the soil was infested with *Fusarium martii phaseoli* and *F. aduncisporum* were infected. Those infected with the latter species were much more dwarfed than those infected with the former. Plants infected with *F. martii phaseoli* from California were much more seriously affected than were those infected with this same species from New York. This may be due to the fact that the former was isolated more recently. That this may be the explanation is supported by a recent work of Burkholder,[7] who found that *F. martii phaseoli* had lost considerable of its virulence after growing in culture for five years.

An experiment was conducted to test the parasitism of *Fusarium aduncisporum* on Davis White Wax beans, and also to determine whether the plants remained susceptible over a period of time. Twenty plants were inoculated by pouring a spore suspension about the base of the plants each week, beginning at about the time when the plants first appeared above ground and continuing for four weeks. Plate 3, B, shows the stage of decay reached in four of those plants 11, 19, 26, and 33 days after inoculation. One hundred per cent of the plants were infected in all cases, and the fungus was recovered from a number of them. This experiment showed that the plants were apparently as susceptible a month after they appeared above ground as they were at first.

[7] BURKHOLDER, W. H. VARIATIONS IN A MEMBER OF THE GENUS FUSARIUM GROWN IN CULTURE FOR A PERIOD OF FIVE YEARS. Amer. Jour. Bot. 12: 245–253. 1925.



A.—At left, a healthy Henderson Bush Lima bean plant from control pot. At right, two plants from soil inoculated with *Fusarium aduncisporum*. Note the decayed surface of the taproots, the absence of fibrous roots, and the dwarfing. The plant at the right had a few long long fibrous roots near the surface of the soil. × ¼

B.—Davis White Wax bean plants inoculated with *Fusarium aduncisporum*. The blackened roots show the extent of the decay, 11 (*a*), 19 (*b*), 26 (*c*), and 33 (*d*) days after they were inoculated

SUMMARY

Root rot of the bean is a very widespread disease in California, where every variety grown commercially is attacked.

Isolations were made from a large number of diseased roots and the fungi obtained were studied. These studies showed that two species of Fusarium capable of causing root rot of the bean occur there. The one species is identical with *Fusarium martii phaseoli* Burk., while the other differs from any hitherto described species of Fusarium. For the latter the name *Fusarium aduncisporum* is proposed, and a description of it has been given herein.

The results of a comparison of *Fusarium martii phaseoli* and *F. aduncisporum* on different culture media are given. Those two species resemble each other very closely on certain media, but differ considerably on others. In general, *F. martii phaseoli* forms more zinc-green and dusky green-blue color, while *F. aduncisporum* forms more cinnamon-brown to clay color.

Both species are about equally parasitic.

A LABORATORY STUDY TO DETERMINE THE BEST TIME TO ENSILE CORN, SUNFLOWERS, AND SUDAN GRASS[1]

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INTRODUCTION

A large number of investigations have been conducted on the various changes that take place during ensiling, and the products and mechanism of fermentation. All these investigations have recognized that there are losses in the constituents of the dry matter during fermentation. However, the stage of maturity at which to cut for ensiling has been selected more or less at random, and the material studied in most instances has been ensiled in large farm silos. Furthermore, the large majority of the investigations have used corn silage only as the material for study.

The investigations reported in this paper are studies of the chemical composition, keeping quality, changes during fermentation, and losses in feed materials during ensiling of three different silage crops, with the aim of selecting more closely the best stages of growth at which to cut them for ensiling. The crops selected for study were corn, sunflowers, and Sudan grass.

PREVIOUS INVESTIGATIONS

Sudan grass, though not widely used as a silage crop, is claimed by Madson and Kennedy (*11, p. 200–201*)[2] of the California Experiment Station to have the characteristic of drought resistance which would make it very valuable in parts of the United States where long dry spells occur during the growing season. It is recommended as a silage crop by Francis and Friedemann (*8*) of the Oklahoma Experiment Station, and by C. H. Eckles (*7*), formerly of the Missouri Experiment Station.

That the sour taste and smell produced when a green crop has been properly ensiled is due to the presence of both volatile and nonvolatile organic acids has been determined by numerous investigators. Dox and Neidig (*4*) at the Iowa station, working with corn silage, state that the sources of the acids are the carbohydrates; and later Neidig (*12*) at the same station shows that the soluble sugars decrease gradually and nearly disappear as the acids increase in amount. Practically all these investigations of volatile and nonvolatile acids in silage have been done on silage selected at one stage of growth only, and have been continued throughout the whole fermentation period. So far as can be discovered, only one investigator has determined the acidity of silage cut at successive periods during the growing season. Blish (*2, p. 14–16*), at the Montana Experiment Station, determined the total acidity of silage from sunflowers cut for ensiling at four successive stages. As far as the writers know,

[1] Received for publication Aug. 4, 1925; issued February, 1926.
[2] Reference is made by number (italic) to "Literature cited," p. 332.

he is the only one who has actually ensiled a crop at several successive stages in order to discover the proper time at which to cut it for ensiling. In addition to acidity, Blish also determined dry matter, ash, protein, ether extract, fiber, and sugars; and from the silage he determined mannitol and alcohol in addition. He obtained good silage at all stages if the juice was allowed to escape or if the plant was allowed to wilt before ensiling.

Small glass containers holding 2 kilos or less have been used successfully for laboratory silos by Lamb (*10*) and Dox and Yoder (*5*) at the Iowa Experiment Station, and by Hunter (*9*) at the Kansas Experiment Station.

Large wooden tubs holding from 200 to 1,000 pounds have been used successfully for experimental silos by Cooke and Hills (*3*, *p. 96–98*), at the Vermont Experiment Station, by Eckles (*6*, *7*) at the Missouri Experiment Station, by Neidig (*13*) at the Idaho Station, and by Westover and Garver (*16*) of the Office of Forage Crop Investigations, Bureau of Plant Industry, United States Department of Agriculture.

THE EXPERIMENTAL WORK

PLAN OF THE EXPERIMENT

The experimental work was divided into three different parts: First—Sudan grass was cut in the field at several successive stages of development and analyzed, to complete the studies of the growing plant begun in a previous paper (*14*) to determine the proper stage of maturity for ensiling. Second—Corn and sunflower plants were ensiled at several successive stages of growth in small laboratory silos to determine by study of some of the products of the silage fermentation, when to cut them for ensiling. Third—Corn, sunflowers, and Sudan grass were each ensiled at four successive stages of growth in large wooden tubs, and analyzed before and after ensiling to determine through a study of the keeping quality, chemical composition, and comparative losses of feed materials the stage at which to cut them to produce the best silage with the smallest loss of feed constituents.

FIELD METHODS

The crops were grown in a section of the field at the Beltsville (Maryland) Experiment Farm usually devoted to silage corn. The preparation of the soil, the planting, and cultivating were done under the supervision of T. E. Woodward, farm superintendent.

The sunflower plants were of a variety known as Giant Russian, and the corn was Boone County White. The sunflowers thrived well in the soil (Bibb silt loam), reaching a height in many cases of 10 to 12 feet.

The Sudan grass seed was obtained through the courtesy of the Office of Forage Crop Investigations, Bureau of Plant Industry, United States Department of Agriculture. The grass grew luxuriantly to a height of 6 feet on the well-drained portion of the field, though it was shorter and ragged in the lower parts of the field.

Representative samples were cut at eight different stages in the development of the plant, using first the height and later the stage of development of the flower and fruit as a guide. Each sample was

wrapped in a waterproof cloth and taken to the laboratory, where it was cut into 1-inch pieces in a hand feed cutter.

The material selected for ensiling in the small laboratory silos, both corn and sunflower plants, was collected and prepared for the silos by the procedure outlined for the Sudan grass.

The corn plant was cut for ensiling at nine successive stages, and the sunflower plant at six successive stages.

The corn used for filling the wooden-tub silos was from selected rows in the regular field of silage corn at the Beltsville Experiment Farm. The sunflowers were from rows planted in the same field. The Sudan grass was grown on a plot about 1 acre in size, sown at one side of the same field.

The stages of maturity of the corn were selected for the tub silos according to the proportion of ears that were in the milk and dough stages. In making the selection a survey was made of a complete row across the field by counting and classifying the ears and selecting that portion of the row which showed the desired proportion of ears in the milk and dough stages. To obtain the desired quantity of samples, about three parallel adjacent rows were combined with the inspected row, on the assumption that they would approximate the maturity condition of the first row.

The stages of maturity selected were (1) 20 per cent dough and 80 per cent milk, (2) 40 per cent dough and 60 per cent milk, (3) 60 per cent dough and 40 per cent milk, (4) 80 per cent dough and 20 per cent milk.

The stages of maturity of the sunflowers were selected according to the development of the flower head. The method of field selection was to examine each individual plant and select for cutting those which were at the desired stage of maturity.

The stages of maturity of the Sudan grass were selected according to the development of the flower and seed. In the field selection it was found that a large area would be in about the same stage of maturity at the same time, so it was possible, by waiting the proper interval, to obtain for cutting a large enough sample for ensiling at successive stages.

SILOS USED

Two different types of silos were used, each for a different phase of the work. For studying the loss of food material during ensiling, small wooden tubs 2 feet in diameter were used. Pressure of 100 pounds per square foot, to approximate that within a large silo, was obtained by levers and weights applied to 2-inch wooden followers fitted as tightly as practicable within the silo walls. For studying phases of the fermentation during ensiling, 1-gallon glass museum jars were used. These were 12 inches deep and 5 inches in diameter, with cover and rubber gasket. A hole of the size to take a No. 5 rubber stopper was ground through the jar cover, and an inverted U-shaped glass tube was inserted through the stopper. The outer end of the tube was immersed in metallic mercury contained in a crucible fastened on the cover with sealing wax. This served as a valve to let out any gas developed and to prevent air getting into the silo.

FILLING THE SILOS

The chopped green material was sampled for analysis as delivered from the hand feed cutter. It was then packed into the jar silo by means of a lever and plunger so that a minimum of air spaces would be left. The gasket and cover were clamped on, the joints coated with paraffin, and the jars stored at room temperature. The jars were weighed before and after filling to obtain the weight of the ensiled material.

After several months the jars were opened in the order filled, and were reweighed to obtain the weight of the silage. Each silage was inspected to determine its quality. After being thoroughly mixed, a sample was taken for analysis.

In preparation for the wooden-tub silos, the silage crop was put through the farm silage cutter, caught in sacks, weighed, packed tightly in the silo, and weighted down. The green crop in the silos was divided into two portions by a large piece of cheesecloth spread over and tucked in 16 inches below the top of the silo. The part below was called the experimental portion, and was used for scoring and analysis. The part above was called the filler, and served as a plug to prevent any air getting to the experimental portion.

After several months the silos were opened, the silage examined and scored, and a sample taken for analysis.

METHODS OF ANALYSIS

One-kilo subsamples of both green crop and silage were dried to air-dry weight in a steam-heated drying closet at about 55° C., and when dry were ground in a power pulverizing mill to pass a 40-mesh sieve. These were used for the determination of moisture, ash, ether extract, and crude fiber. The remainder of the moist samples were ground to a wet pulp in a power meat chopper and sampled to determine dry matter and proteins. In cases where sugars or acidity were to be determined, a portion of the wet pulp was used to make an alcoholic extract.

Dry matter was determined by drying the sample to constant weight in a water-jacketed vacuum oven at the temperature of boiling water. Protein, ash, ether extract, and crude fiber were determined by the methods outlined in the Methods of Analysis of the Association of Official Agricultural Chemists (*1, p. 71–99*). Nitrogen-free extract was calculated by difference.

The alcoholic extract of the wet pulp was prepared according to the method of Swanson and Tague (*15*). Total and reducing sugars were determined on aliquots of this extract by the gravimetric cuprous-oxide method of Walker and Munson (*1, p. 71–99*).

Total acidity was determined on four 100 c. c. aliquots of the alcoholic extract with N/20 sodium-hydroxide solution, using phenolphthalein as an indicator.

Volatile acidity was determined as follows: The aliquot portions of the extract titrated for total acidity were combined to make two samples, each corresponding to 200 c. c. of the original extract. An excess of alkali was added, and the samples were evaporated nearly to dryness. Each was then washed into a 500 c. c. side-neck distilling flask and made up to 300 c. c. with distilled carbon-dioxide-

free water, and a sufficient quantity of normal sulphuric acid was added to release the organic acids. The contents of the flask were then distilled in a current of steam at atmospheric pressure, using carbon-dioxide-free water in the steam flask. Four liters of distillate were collected and titrated with N/20 sodium-hydroxide solution, using phenolphthalein as an indicator.

Volatile acidity was expressed as percentage of acetic acid and was calculated on the basis of the moist silage. The percentage figures for nonvolatile acidity were expressed as lactic acid. They were obtained by subtracting the cubic centimeters of tenth-normal sodium hydroxide consumed by the volatile acid in the 40-gram charge of silage from that consumed by its total acid content, and calculating the difference as percentage of nonvolatile or lactic acid.

The figures for nonreducing sugars were obtained by subtracting figures for reducing sugars from those for total sugars.

DISCUSSION OF RESULTS

THE GROWING SUDAN GRASS

Table I, giving the chemical composition of the Sudan grass plant at eight successive stages of growth is presented in full to complete the series corn-sunflower-Sudan grass. In addition to the analyses made on the corn and sunflower plants, determinations for ash, ether extract, crude fiber, and nitrogen-free extract were made on the Sudan grass samples.

TABLE I.—*Composition of Sudan grass plant at successive stages of growth*

Stage of maturity	Dry matter in fresh material	Water-free basis							
		Total protein	Albuminoid protein	Ash	Ether extract	Crude fiber	Reducing sugar	Nonreducing sugar	Nitrogen-free extract by difference
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Two feet high and under	15.5	20.2	15.6	7.4	2.6	24.2	7.3	0.7	45.7
Three to four feet high	13.9	15.5	12.8	7.4	2.4	26.0	11.3	2.1	48.7
Four to six feet high	20.0	8.3	7.7	4.5	1.5	29.8	17.4	2.3	56.0
Early blooming stage	22.3	8.1	6.7	3.9	1.2	30.6	16.0	1.1	56.2
Middle blooming stage	34.2	5.0	3.8	4.4	1.1	33.7	11.0	1.5	58.8
Early dough stage	36.9	4.8	3.7	4.3	1.1	33.7	10.2	.7	56.2
Advanced dough stage	37.7	4.8	4.0	4.7	1.3	33.4	10.3	.3	55.8
Seed mature	41.3	5.3	4.6	4.8	1.2	33.4	5.6	.3	55.4

The chemical composition alone makes a somewhat unsatisfactory basis for selecting the stage of growth at which to cut a plant for ensiling. Very early stages can not be selected, even if the dry matter is high in feed material, because the yield per acre is too low to be profitable and the moisture is so high that the silage will be too wet. Neither can a very late stage be selected, though the yield per acre of dry matter is high and the amount of material is large. At these stages the moisture is so low and the crude fiber so high that unless water is added during the ensiling process the crop will not pack well in the silo and the silage will be dry and unpalatable. A selection must be made with reference to total yield, dry-matter content, succulence, and percentage of feed material.

By the time the Sudan grass had reached the early blooming stage it had probably reached its full height and diameter of stalk. The development of seeds, due to their small size, was probably not attended by any great increase in amount of feed material. It is quite true that the moisture content was high in the early blooming stage, but it was not unreasonably so. While some of the feed constituents expressed as percentages of the water-free material were lower in the early blooming stage than in some of the preceding stages, yet there was doubtless more actual material per acre and not so much danger of the silage being too wet. On the other hand, the Sudan grass at the later or middle blooming stage was probably too low in moisture to pack well in the silo or to produce a succulent silage, and was lower in percentage of feed constituents in the water-free material than it was at the early blooming stage.

It would seem reasonable, therefore, on the basis of this study, to select the early blooming stage as the best stage at which to cut Sudan grass for ensiling.

THE CORN AND SUNFLOWER PLANTS ENSILED IN LABORATORY SILOS

The weight of the corn and sunflower plants ensiled in small laboratory silos at several successive stages of growth was about 2 kilos in every silo. The silage in every silo was of excellent quality, having an agreeable taste, odor, and appearance. There was a progressive development of silage odor and taste from the silages made during the earlier stage of plant development to those made when the crops were mature. Tables II and III give the percentages of sugars in green plants as ensiled, and percentages of sugars and acids in the resulting silages.

TABLE II.—*Corn: Sugars in green plants as ensiled and sugars and acids in the resulting silage*

Stage in growth of the plant	Dry matter in green crop	Water-free basis				Moist basis	
		Reducing sugar		Nonreducing sugar		Volatile acid as acetic	Nonvolatile acid as lactic
		Into silo	Out of silo	Into silo	Out of silo		
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
3 feet high	15.8	14.69	Trace.	2.73	0.38	0.48	1.18
4½ to 5 feet high	14.9	16.69	None.	3.23	None.	.65	.84
Just tasseling	18.3	13.13	Trace.	1.85	.14	.52	1.33
Just silking	18.4	18.23	1.71	1.30	.24	.52	2.50
Kernels forming	18.8	20.37	[a]11.86	5.44	.13	.47	1.95
Milk stage	22.4	17.59	3.50	4.51	.46	.63	1.06
Silage stage, half milk, half glazed	31.3	10.23	2.28	2.81	.09	.55	10.5
All glazed	35.8	8.50	1.92	5.39	.13	.79	.78
Ready to shock	40.2	7.71	1.97	2.73	.03	52	1.00

[a] Here is an apparent discrepancy which the writers do not attempt to explain. This figure is an average of duplicates on the same sample—11.91 per cent and 11.81 per cent. The corresponding percentages of total sugar were 12.18 and 11.79.

TABLE III.—*Sunflower: Sugars in green plants as ensiled and sugars and acids in the resulting silage*

Stages in growth of the plant	Dry matter in green crop	Water-free basis				Moist basis	
		Reducing sugar		Nonreducing sugar		Volatile acid as acetic	Nonvolatile acid as lactic
		Into silo	Out of silo	Into silo	Out of silo		
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
3 feet high	10.5	13.12	None.	1.90	None.	0.50	0.11
6 to 7 feet high	11.5	15.62	None.	.78	None.	.65	.16
Just budding	14.0	16.16	Trace.	1.54	Trace.	.66	.42
In full bloom, seeds small	18.6	11.73	Trace.	1.99	None.	----------	----------
Early dough stage, seeds half grown	17.7	16.88	0.74	3.23	0.45	.45	.89
Middle dough stage	22.3	6.68	.45	.63	.36	.77	.73

The green crops in both corn and sunflower showed an increase in the percentage of reducing sugars from that found in the first silo to a maximum, and then declined again as the seeds matured. In the corn the maximum occurred at the time the kernels were forming. The reducing-sugar content of the corn silage at that stage was unusually high. In the sunflower plant the maximum percentage of reducing sugars occurred at the early dough stage when the seeds were half grown. The loss of reducing sugars in the silo was complete in the first three stages, but in the later stages there were some sugars left in the silage. More than 50 per cent of the reducing sugars present in the corn ensiled when the kernels were forming were present in the corresponding silage.

The percentages of reducing sugars in the sunflower plant were lower than in the corn plant, and the sugars were more completely used up in the corresponding silage.

The percentage of the nonreducing sugars in the green plant was in all cases much less than that of the reducing sugars, and in the corresponding silages only traces or none at all remained after the fermentation had ceased.

The percentage of volatile acidity in the corn silage varied from 0.47 to 0.79 per cent of the moist silage, with no definite increase or decrease anywhere. The percentage of nonvolatile acidity increased from that found in the silage at the 3-foot stage to a maximum of 25 per cent at the silking stage, and then decreased gradually. At the silking stage, the nonvolatile acidity was about five times the volatile acidity. The maximum nonvolatile acidity was at the same stage of growth as the maximum total acidity.

The variation of volatile acidity in the sunflower silage was only from 0.45 to 0.77 per cent of the moist silage. The volatile acidity averaged considerably higher than the nonvolatile. The percentage of nonvolatile acidity was low in the early stages, and gradually increased. The total acidity in the sunflower silage, which reached a maximum at the early dough stage, was much lower than that in the corn silage.

If the percentages of sugars and acidity were to be used as the sole basis for selecting the best stage at which to cut forage crops for ensiling, it would be natural to select the period of maximum per-

centage of these constituents, because at this time the silage would be most safely preserved. The maximum percentages of sugars and acidity in both corn and sunflowers, especially in corn, seemed to be when the seeds were small and watery, or at least not more than half grown, and when the water content of the plant was still somewhat high.

It is concluded from the examination of the silage in the laboratory silos, and study of Tables II and III, that while it is necessary to have in a silage crop a sufficient amount of fermentable sugars, and in the silage enough organic acids to prevent spoilage, data for these factors taken alone are insufficient for determining the proper time for ensiling a green crop.

THE CORN, SUNFLOWER, AND SUDAN GRASS ENSILED IN TUB SILOS

The weights of corn, sunflower, and Sudan grass plants before and after ensiling are given in Table IV. With the aid of the figures given in the table, the weights of the different chemical constituents before and after ensiling and their changes in amount may be calculated and any apparent discrepancies between tables explained.

TABLE IV.—*Weights of corn, sunflower, and Sudan grass plants before and after ensiling (experimental portion)*

Silo No.	Corn plant		Sunflower plant		Sudan grass plant	
	Into silo	Out of silo	Into silo	Out of silo	Into silo	Out of silo
	Pounds	*Pounds*	*Pounds*	*Pounds*	*Pounds*	*Pounds*
1	286.0	277.5	244.5	241.0	216.5	208.1
2	310.0	303.0	374.0	356.0	158.5	157.2
3	275.5	269.5	318.0	311.75	167.0	
4	277.0	262.5	240.5	230.5	149.0	148.5

THE CORN PLANT

The corn silage from all four silos was excellent in quality, though next to the staves there was a woody odor. If there was any first choice among the silages it was the silage cut at stage 4 (Table V).

TABLE V.—*Composition of corn plant before and after ensiling (experimental portion)*

Silo No.	Stage of maturity of corn plant	Dry matter		Water-free basis					
				Albuminoid protein		Crude fiber		Nitrogen-free extract	
		Into silo	Out of silo	Into silo	Out of silo	Into silo	Out of silo	Into silo	Out of silo
1	20 per cent dough, 80 per cent milk	*Per cent* 27.90	*Per cent* 25.85	*Per cent* 6.90	*Per cent* 5.33	*Per cent* 20.71	*Per cent* 21.29	*Per cent* 66.01	*Per cent* 63.43
2	40 per cent dough, 60 per cent milk	32.36	31.15	5.89	4.32	21.40	18.24	66.35	69.40
3	60 per cent dough, 40 per cent milk	33.69	34.14	6.30	4.60	20.48	20.51	66.05	66.61
4	80 per cent dough, 20 per cent milk	31.20	32.89	5.63	4.44	23.16	20.50	64.67	66.62

Throughout all the four stages of growth the percentage composition of both the corn plant and corn silage showed very small changes. Stage 3 was highest in/ dry matter; stage 1 was highest in albuminoid protein; stage 2 was highest in nitrogen-free extract and lowest in crude fiber. Since the dry matter in stage 3 was somewhat higher than is usually thought desirable, the figures given in Table V seem to indicate that the corn plant in stage 2, when 40 per cent in the dough and 60 per cent in the milk was at the best stage to cut for ensiling.

However, the figures given in Table VI for loss in feed constituents during ensiling showed very conclusively that the corn plant ensiled when the kernels are 80 per cent in the dough and 20 per cent in the milk suffered the least loss of feed constituents. This fact, combined with the scoring of the silage on opening the silos, indicated that the best stage of the four tried out was when the kernels were 80 per cent in the dough and 20 per cent in the milk.

TABLE VI.—*Gain (+) or loss (−) in feed constituents of corn silage (based on weight of constituent ensiled)*

Silo No.	Stage of maturity of corn plant	Dry matter	Albuminoid protein	Crude fiber	Nitrogen-free extract
		Per cent	*Per cent*	*Per cent*	*Per cent*
1	20 per cent dough, 80 per cent milk	−10.2	−30.6	−7.7	−13.7
2	40 per cent dough, 60 per cent milk	−5.8	−30.9	−19.7	−1.4
3	60 per cent dough, 40 per cent milk	−.9	−27.6	−.7	+.03
4	80 per cent dough, 20 per cent milk	−.02	−21.4	−11.7	+2.8

THE SUNFLOWER PLANT

Like the corn plant, the sunflowers in all four stages produced good silage. The first three had a very noticeable sunflower-oil odor, which, however, was least noticeable in the silage made at stage 3. Silages made at stages 1 and 2 lost moisture by seepage through the silo bottom. The silage from stage 4 was somewhat dry and woody, but this silage had the cooked odor thought desirable in the best silage. As no palatability test was conducted, it was entirely a matter of opinion whether the silage from stage 3 or stage 4 would be more relished by cows.

According to Table VII, the dry matter is highest in the silage from stage 4, and the albuminoid protein and the nitrogen-free extract highest and the crude fiber lowest in the silage from stage 1.

On the basis of the chemical composition, the best stage at which to cut the sunflowers for ensiling was when they were in full flower, before the rays had begun to wilt or fall.

TABLE VII.—*Composition of sunflower plant before and after ensiling (experimental portion)*

Silo No.	Stage of maturity of sunflower plant	Dry matter		Water-free basis					
				Albuminoid protein		Crude fiber		Nitrogen-free extract	
		Into silo	Out of silo	Into silo	Out of silo	Into silo	Out of silo	Into silo	Out of silo
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	In full flower	20.78	20.71	6.84	4.75	26.42	31.78	54.27	48.58
2	Rays wilted, but not fallen	19.51	20.96	5.64	3.98	31.89	33.83	50.84	45.64
3	Rays dry, partly fallen	20.93	22.16	6.22	3.57	37.38	35.08	44.93	41.41
4	Rays dry, all fallen	22.10	24.49	6.09	3.90	36.92	34.68	44.60	41.18

According to Table VIII, the silage from all except stage 1 had apparently gained in dry matter. Of the three silages which apparently gained in dry matter, that from stage 4 gained the largest percentage. The silage from stage 2 showed the least loss of albuminoid protein, that from stage 4 showed the least loss of nitrogen-free extract, and that from stage 1 showed the largest loss of crude fiber. With reference to loss of feed constituents, the silage from one stage rated as well as that from another.

TABLE VIII.—*Gain (+) or loss (−) in feed constituents of sunflower silage (based on weight of constituent ensiled)*

Silo No.	Stage of maturity of sunflower plant	Dry matter	Albuminoid protein	Crude fiber	Nitrogen-free extract
		Per cent	*Per cent*	*Per cent*	*Per cent*
1	In full flower	−2.0	−31.9	+15.3	−14.3
2	Rays wilted, but not fallen	+2.5	−27.7	+8.4	−8.0
3	Rays dry, partly fallen	+3.6	−40.6	−2.8	−4.5
4	Rays dry and all fallen	+6.4	−31.8	−.1	−1.8

According to appearance and apparent palatability, the sunflowers were in the best stage for ensiling when the rays were dry and either partly fallen or all fallen. According to chemical composition, the best stage for ensiling was earlier, when the plant was just in full flower. And judging by the loss in feed constituents, the sunflowers were ready to cut when the rays were wilted but not yet fallen.

No feeding experiment or palatability test was run at the time of these ensiling experiments. However, it is probable that sunflowers cut at any one of the four stages of growth would produce silage satisfactory for feeding.

THE SUDAN GRASS

The Sudan grass silage made at the first two stages was very good, but was not as fragrant as corn silage. The silages made at the last two stages were too dry to be called good silage. The fermentation seemed normal in both these, but the Sudan grass had been too dry and strawlike to pack well.

According to Table IX, Sudan grass had the largest percentage of dry matter at stage 4, the largest percentage of albuminoid protein and the smallest percentage of crude fiber at stage 1, and the largest percentage of nitrogen-free extract at stages 3 and 4. Since the Sudan grass at stages 3 and 4 made somewhat unsatisfactory silage, the selection would be between the silages from stages 1 and 2. On the basis of the percentage composition, either one could be selected as the best stage at which to ensile Sudan grass.

TABLE IX.—*Composition of Sudan grass before and after ensiling (experimental portion)*

Silo No	Stage of maturity of Sudan grass	Dry matter		Water-free basis					
				Albuminoid protein		Crude fiber		Nitrogen-free extract	
		Into silo	Out of silo	Into silo	Out of silo	Into silo	Out of silo	Into silo	Out of silo
		Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
1	Early blooming stage	26.01	25.00	5.53	3.93	31.95	32.17	52.90	52.45
2	Middle to late blooming stage	29.89	30.70	4.73	3.19	31.49	32.69	55.37	54.19
3	About dough stage	35.35	30.70	4.27	2.98	33.53	34.85	54.50	52.94
4	Advanced dough stage	37.42	33.36	4.15	3.24	33.67	35.83	55.43	51.96

Of the two first stages in Table X, the silage at stage 2 lost less dry matter and nitrogen-free extract, and the silage at stage 1 lost less albuminoid protein and more crude fiber.

TABLE X.—*Gain (+) or loss (−) in feed constituents of Sudan grass silage (based on weight of constituent ensiled)*

Silo No.	Stage of maturity of Sudan grass	Dry matter	Albuminoid protein	Crude fiber	Nitrogen-free extract
		Per cent	*Per cent*	*Per cent*	*Per cent*
1	Early blooming stage	−7.6	−33.6	−7.0	−8.5
2	Middle to late blooming stage	−4.4	−35.6	−.7	−6.4
3	Dough stage *				
4	Advanced dough stage	−11.2	−30.7	−5.3	−16.7

* Figures not available.

Thus, on the basis of appearance, chemical composition, or loss in feed constituents, Sudan grass cut at either the early blooming stage or the middle to late blooming stage made a very satisfactory silage.

GENERAL CONCLUSIONS

It would seem from a study of this series of tables that the content of fermentable carbohydrates is sufficient to produce the proper degree of acidity in the silage from any green crop—except a few very exceptional cases, such as reported by Blish (*2, p. 14–16*) at the Montana Experiment Station. The only other chemical qualification necessary to a succulent silage is the proper moisture content.

Therefore, almost any forage crop which is palatable as a forage crop will be palatable as silage if the moisture content is right. If

the moisture content is too high the silage will be water-logged, or soluble feed materials will be lost by drainage. If the moisture content is too low and water is not added, the silage will not be succulent, and will not pack well enough to prevent the leakage of air and the growth of molds and undesirable fermentations.

SUMMARY

A study of the chemical composition of the growing plant showed that the best time to cut Sudan grass for ensiling was at the early blooming stage.

A study of the percentage of sugars in the growing corn and sunflower plants and the percentage of organic acids in the resulting silage, showed that the sugars and acids taken alone provided an insufficient basis on which to select the proper stage for ensiling the green crop.

It was concluded from the study of the growing plant and the resulting silage that of the stages selected the best one at which to cut the corn plant for ensiling was when 80 per cent of the kernels were in the dough stage and 20 per cent in the milk stage.

From a similar study of the sunflower plant no definite conclusion could be reached. Sunflowers cut at any one of the four selected stages produced satisfactory silage.

A study of the growing plant and the resulting silage showed that Sudan grass cut at either the early blooming stage or the middle to late blooming stage made very satisfactory silage.

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FURTHER STUDIES ON THE OVERWINTERING OF PSEUDOMONAS CITRI[1]

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INTRODUCTION

An opportunity was afforded to study certain phases of the overwintering of *Pseudomonas citri* Hasse in connection with the investigations on the susceptibility and resistance of Citrus plants to canker, in the isolation field in southern Alabama, during the years of 1917 to 1921. To a large extent these studies were confined to the overwintering of the organism under natural conditions within the tissues of the host plant, as this is by far the most important, if not the only, way that overwintering of *P. citri* occurs.

Owing to the many types of plants growing in the field, their individual behavior to environmental conditions could be closely noted and correlated with their susceptibility during the growing season and the overwintering of the canker organism. The winters of 1919–20 and 1920–21 were normal ones in most respects for southern Alabama. The first was characterized by freezing temperatures the fore part of March; the second by killing frosts early in November. In both instances temperatures low enough and of sufficient duration occurred to kill the young leaves of the Citrus plants in the spring of 1920, and caused or hastened the defoliation of the mature leaves in November of the same year. Lower temperatures were recorded during the season of 1918–19, while the winter of 1917–18 was rather severe. The variety of these winter seasons encountered in the field made possible a rather comprehensive study of the overwintering of *Pseudomonas citri.*

MEANS OF THE OVERWINTERING OF THE ORGANISM

The Citrus canker organism may possibly overwinter in one of the following four ways:

(1) In the soil.

(2) In spots on defoliated leaves.

(3) In old diseased spots on the leaves attached to the trees, together with cankers on the twigs and larger limbs.

(4) By an extended or arrested incubation period.

IN THE SOIL

Numerous attempts to isolate *Pseudomonas citri* directly from orchard soil failed. The senior writer spent the greater part of two seasons making isolations from soil collected under many and widely

[1] Received for publication July 2, 1925; issued February, 1926. Published with the approval of the director of the Alabama Agricultural Experiment Station. The paper is based on cooperative investigation between the Office of Crop Physiology and Breeding Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Department of Plant Pathology, Alabama Agricultural Experiment Station. This investigation was completed June 30, 1921.

divergent conditions in the field with negative results. D. C. Neal and Julius Matz, both formerly connected with the work, and the junior author of this paper continued these efforts to isolate the organism from the soil. All attempts yielded negative results, except in one instance when Mr. Neal was able to isolate the organism from soil taken from under a badly infected grapefruit tree directly after a heavy rain.

Our negative results can be readily explained in the light of the work of Fulton [2] and Lee.[3] Both investigators found that *Pseudomonas citri* underwent a rapid and continuous decline in numbers in all the types of soil which they tested, and that the vanishing point was reached in about two weeks. The writers in their present study were attempting to recover the organism from soil on which infected trees had been destroyed a month to three years previously. From their failure to recover the organism from the soil after infected trees were removed, and from the fact that both Fulton and Lee found that it disappears in soil after an interval of two weeks, the writers conclude that *Pseudomonas citri* can not survive for any length of time in the soil, and that the soil does not afford a means for the organism to live over winter.

IN THE SPOTS ON DEFOLIATED LEAVES

The possibility of *Pseudomonas citri* passing the winter in canker spots on defoliated leaves was pointed out earlier by Wolf,[4] who stated:

> Old leaves on the ground may possibly harbor the organism and there it may remain viable for a long time. It is believed, moreover, that the organism survives the winter in fallen leaves and that these fallen leaves constitute a very important source of infection in the following spring.

He further records unsuccessful attempts to isolate the organism from leaves kept in the laboratory from September to May and from twig cankers kept from March to October.

The importance of such a manner of overwintering is readily seen when it is considered that during the winter of 1917–18, and to a less extent in 1918–19, complete defoliation of both infected and uninfected leaves of the two important Citrus fruits grown in Alabama—grapefruit (*Citrus grandis* (L.) Osbeck) and Satsuma (*Citrus nobilis var. unshiu* Swingle)—occurred. *Poncirus trifoliata* (L.) Raf., being of deciduous nature, sheds all of its leaves normally with the approach of cold weather. The winter 1919–20 was normal for this locality and less defoliation occurred; in fact, a large number of infected leaves remained on grapefruit, Satsuma, and trifoliate orange hybrids until after the first growth matured. Thus, weather conditions determined to a great extent the severity of defoliation, not only of healthy but also of infected leaves.

To determine just how long the Citrus canker organisms were able to survive in spots on leaves that had defoliated and fallen to the ground, the following experiments were carried out during the winter of 1919–20. Defoliated and infected leaves were collected from be-

[2] FULTON, H. R. DECLINE OF PSEUDOMONAS CITRI IN THE SOIL. Jour. Agr. Research 19: 207–223. 1920.
[3] LEE, H. A. BEHAVIOR OF THE CITRUS-CANKER ORGANISM IN THE SOIL. Jour. Agr. Research 19: 189–206, illus. 1920.
[4] WOLF, F. A. CITRUS CANKER. Jour. Agr. Research 6: 69–100, illus. 1916.

neath badly diseased grapefruit and citrange plants and each group was divided into three sets—one of which was exposed on the surface of the soil, the second buried 3 to 6 inches in the soil, and the third kept in the laboratory. Isolations were made at weekly intervals from a number of representative spots from each set. Positive results were obtained from three grapefruit spots after two weeks exposure buried in the soil, and from one spot after one week in the laboratory. From citrange leaves *Pseudomonas citri* was isolated from one spot after one week's exposure on the surface of the soil. In no case was the organism recovered after three weeks exposure under any conditions. It is interesting to note in this connection that a rapid disintegration of the buried leaves took place, so that by the end of the third week the identity of the leaf was almost lost. Thus, from these results it may safely be concluded that the life of *P. citri* in defoliated leaves is not of sufficient duration to carry the organism through the winter.

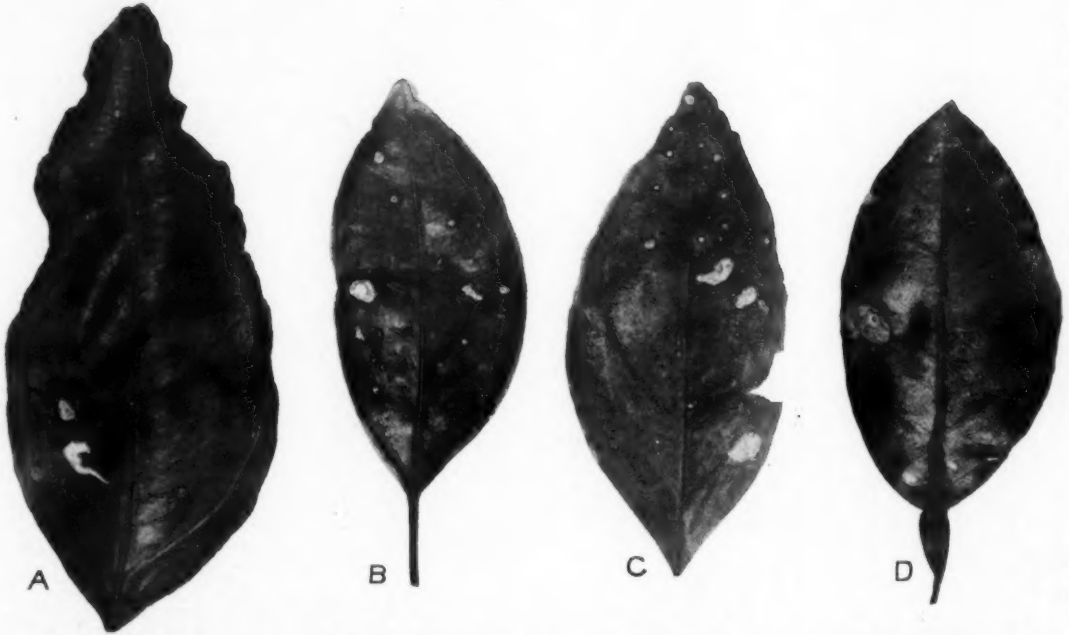
IN OLD SPOTS ON THE LEAVES ATTACHED TO THE TREES, TOGETHER WITH CANKERS ON THE TWIGS AND LARGER LIMBS

The number of canker spots on the leaves which go through the winter depends primarily on the amount of defoliation. During some winters the percentage of defoliation of the infected leaves is very high. Counts made during two winter seasons showed that the percentage of infected leaves which drop was much higher than the percentage of noninfected leaves which drop.

That viable bacteria are present in some of the old spots on the leaves which go through the winter attached to the trees has been shown by repeated isolations of *Pseudomonas citri* made during the winter and spring. Further, around the margin of these old spots many small fresh spots are formed in the spring. A number of such spots are shown in Plate 1 on a number of leaves of different Citrus plants. In the many isolations made, the largest numbers of viable spots were on those leaves which were formed late in the summer or fall.

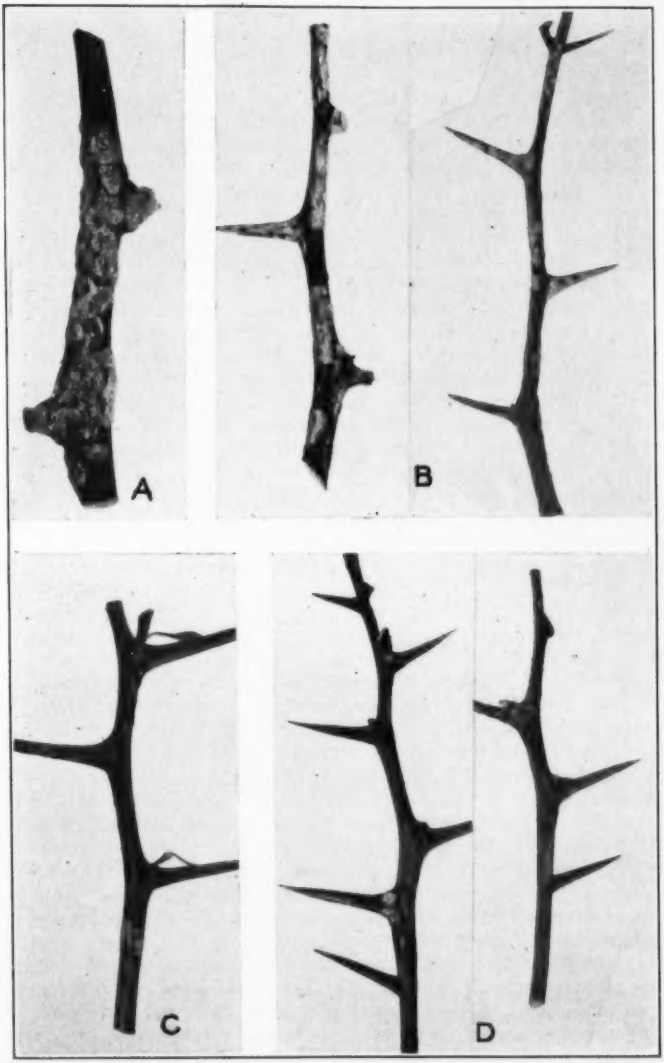
The large number of twig and stem cankers present in the isolation field afforded abundant material for observations and isolations throughout the winter of 1919–20. These cankers were classified according to age, condition, and location on the tree. Taking these in their chronological order, the first are those formed only on citranges early in the spring of 1918 following inoculations made in September of the preceding year. These areas of canker tissue are characterized by their location, usually near the base of the original trunk, by their extremely loose, corky construction, and further by the fact that their margin lacks a definite oily outline. The striking differences between these areas and the cankers formed later in the same season are clearly shown in Plate 2, A, B. Those shown in Plate 2, A, are the result of artificial inoculation, and such as these are dealt with in the overwintering paper by Peltier and Neal.[5] The cankers formed later in 1918 resulted from natural infection, and are more limited in area. These two types of cankers are quite similar.

[5] PELTIER, G. L., and NEAL, D. C. OVERWINTERING OF THE CITRUS-CANKER ORGANISM IN THE BARK TISSUE OF HARDY CITRUS HYBRIDS. Jour. Agr. Research 14: 523–524, illus. 1918.



A.—Satsuma (Ikeda) leaf B.—Satsuma (Owari) leaf C.—Satsumelo leaf D.—Grapefruit (Duncan) leaf

(Photographs taken May 1, 1920, to show the breaking out of many small canker spots at the margins of old spots formed the preceding season. About natural size)



A.—Citrandarin. Cankers formed in the spring of 1918, as a result of inoculations made in September, 1917

B.—*Poncirus trifoliata*. Cankers formed during the summer of 1918 as result of natural infection. Isolations made in the spring of 1920 from cankers, similar to those shown in A and B, were all negative

C.—*Poncirus trifoliata*. Cankers formed during the spring and summer of 1919 as a result of natural infection. A small percentage of spots similar to these were viable in the spring of 1920

D.—*Poncirus trifoliata*. Cankers formed in the fall of 1919 as a result of natural infection. A high percentage of spots of a similar nature were viable in the spring of 1920

(Photographs taken May 1, 1920. All about natural size)

Cankers formed during the growing season of 1919 were quite numerous. For convenience they are classified under two heads—those formed during the spring and summer, and those formed in the fall. The first can easily be distinguished from cankers of the preceding year, by their position on the tree, by their construction, and by general appearance. They are found, in practically all cases, on the wood of the last season's growth which has either rounded or shows some sign of bark formation. These spots are generally ruptured and corky, depending somewhat on the host and time produced, and they range in color from light yellow to brown. A large number of them showed a more or less distinct oily margin late in the fall. Cankers such as these are shown on Plate 2, C. However, with mild weather in the fall, an entirely different type of spot was produced on the twigs of the trifoliate orange and its hybrids and grapefruit; these cankers formed on the last growth of the season ranged in diameter from 1 to several millimeters, were extremely oily (with a reddish-brown color), and remained unruptured during the winter. (This type of canker is clearly shown in pl. 2, D.) An exception must be made to this statement in the case of grapefruit. Owing to the quick response of grapefruit to temperature changes, cankers of this type on angular wood which was not killed by the first low temperature were able to develop from time to time as the host plant became active, and so did not go through the winter in unruptured condition.

Thus, four types (as to age) of twig and stem cankers were present in the fall of 1919. The results of numerous isolations late in the winter of 1919–20 and early in the spring from the above-described cankers showed that practically 95 per cent or more of the cankers with viable bacteria belonged under the type formed late in the summer of 1919. Cankers formed earlier in 1919, although they showed all signs of being active when they went into the winter, gave negative isolations in the majority of cases. Isolations from canker formed the two preceding years were negative in all instances.

The results from the large number of isolations from twig and stem cankers belonging to the various types just described clearly indicate that the life of the bacteria in a canker is more or less limited, and that only some of the cankers formed the preceding season serve as a source for new infections in the spring. In fact, there seems to be a close correlation between the type of wood (angular and round) and spring viability of stem and twig cankers. With possibly only one or two exceptions, spots which went into the winter upon round wood which showed definite bark formation have in all cases yielded negative results upon isolation. The cankers classified as having been formed in 1918 were all dead, while a large per cent of those formed in the early spring and summer of 1919 were no longer viable. Only the spots formed late in the fall of 1919, on angular wood, gave a high percentage of positive results upon isolation throughout the winter and spring.

An interesting observation was made in connection with the cankers of various ages in the spring of 1920. Upon carefully removing the corky tissue from the cankers formed in 1918, it was noted that they were gradually being delimited from the healthy tissue by a pronounced layer of new green bark. The viable spots formed late in 1919, however, extended down through the cambium layer, often discoloring the wood.

BY EXTENDED OR ARRESTED INCUBATION PERIOD

Pure-culture inoculations on citrange, citrange hybrids, and trifoliate orange, grapefruit, and Satsuma were made September 16, 1917. During the remainder of the season only a few canker spots developed on the leaves of the trifoliate orange and grapefruit as the immediate result of these inoculations. Owing to the severe winter, all the infected leaves fell off and no spots of any description were visible on these plants in the spring. However, with the resumption of active growth of the plants in the spring, cankers developed over extensive areas[6] on the stems of the trifoliate oranges and some of its hybrids. Apparently the bacteria were able to gain entrance into the plant tissues, possibly through the lenticels, and by means of an incubation period was carried over until the following spring.

With these results in mind, a careful examination of the plants was made during the following years in order to determine whether anything of this sort occurred under natural conditions. During the winter of 1919–20 and again the following year this method of overwintering was observed to take place on the leaves of Satsuma. In April of 1920 a number of characteristic spots were observed on Satsuma leaves of the last growth of the preceding year. These leaves did not show any signs of spots prior to and during the winter. When they first developed on the leaves they resembled melanose in some respects, and it was only by isolations that the identity of the causal organism was definitely determined. Apparently initial infection took place shortly before the plants went into dormancy, and incubation of the organism was arrested until active growth of the plants was resumed in the spring. As a rule the spots were bun ched, small, very oily, slightly raised on both sides of the leaf, and remained unruptured for some time (pl. 3, A). That these spots were not due to early spring infection was determined by the fact that the first canker spots appeared on Satsuma leaves of the first spring growth May 10, one month later than the others were observed on the mature leaves. All attempts to inoculate leaves formed the preceding fall had failed.

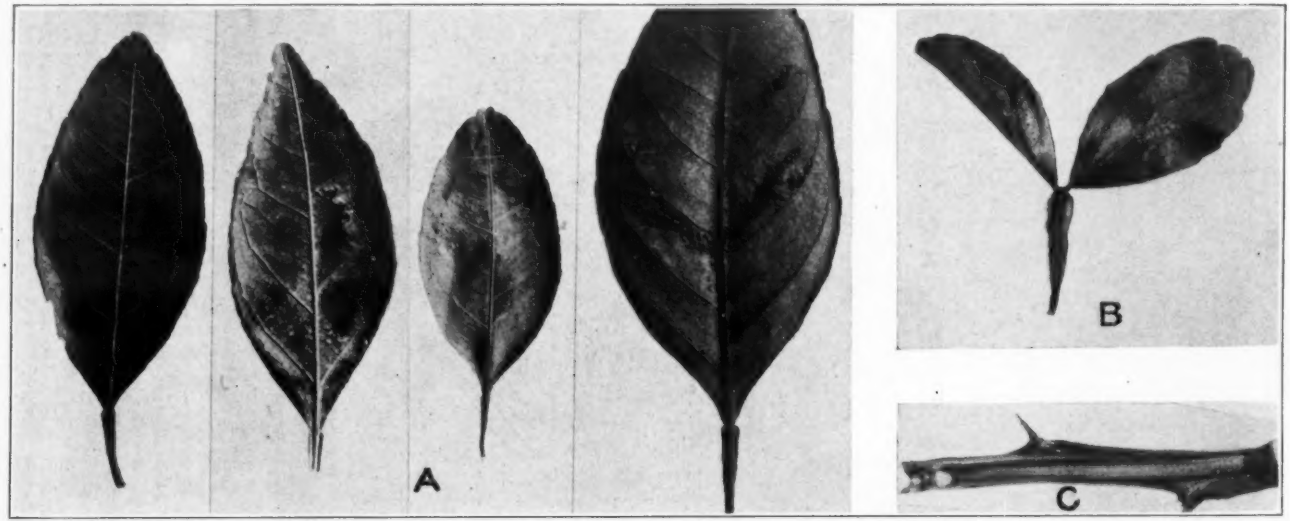
Similar observations were made during the following winter season on Satsuma. Whether such delayed infections occur on other Citrus plants could not be determined, in spite of the many observations made.

SOURCES OF EARLY SPRING INFECTION

The sources of early spring infection varies from season to season. Since this paper is primarily concerned with the overwintering of *Pseudomonas citri* within the tissues of the host, a general statement of the condition of the plants, the amount of infection present, and the weather conditions during the winter are necessary.

The winter of 1917–18 was the most severe to which the plantings in the isolation field were subjected. A minimum temperature of 15.5° F. was recorded in January, and only the more hardy plants survived, while complete defoliation of all plants resulted. With

[6] PELTIER, G. L., and NEAL, D. C. OVERWINTERING OF THE CITRUS-CANKER ORGANISM IN THE BARK TISSUE OF HARDY CITRUS HYBRIDS. Jour. Agr. Research 14: 523–524, pl. 58. 1918.



A.—Typical specimens of Satsuma leaves, showing the nature of the arrested incubation period. These leaves were formed late in 1919, infected naturally by *Pseudomonas citri* before they matured. Owing to adverse environmental conditions, the organism failed to develop, but remained quiescent until the following April, when characteristic canker spots were formed

B.—Citrandarin leaf, showing new spots developing at margin of spot formed the preceding season

C.—Grapefruit twig, showing the renewed activity of *Pseudomonas citri* at the base of the leaf scars

(Photographs taken May 1, 1920. All about natural size)

the exception of a few leaf spots on grapefruit and trifoliate orange, none of the other plants showed any visible lesions of citrus canker when they went into dormancy. Consequently, the only viable organisms were those which produced cankers in the early spring in the bark tissues of the trifoliate orange and some of its hybrids.[7] These organisms overwintered within the tissues by extended or arrested incubation period.

During the summer of 1919, all the plants were in an excellent condition, having made a good and abundant growth. Consequently, the more susceptible plants were heavily infected. Leaf, twig, and stem cankers were numerous. The autumn was unusually mild and active growth of grapefruit and Satsuma plants continued until well into November. Thus, the cankers were able to continue development and also new infections occurred.

After a normal winter, such as that of 1919–20, two distinct sources of spring infection were recognized—viable spots on the leaves and twigs formed the previous year, and through the extended incubation period of the organism on leaves infected late in the fall.

The winter of 1918–19, while not as severe as that of the preceding year, caused considerable defoliation. The weather records of the four years previous showed that the winter of 1918–19 could be classed as intermediate between the severe winter of 1917–18 and the normal winter of 1919–20. In fact, the monthly mean temperature, and the number and severity of frosts for the winter of 1918–19 and 1919–20 varied but little. However, a minimum temperature of 17° F. was recorded in January, 1919, which was low enough to cause complete defoliation of practically all of the plants in the field. The lowest temperature reached during th winter of 1919–20 was 25° F. A heavy infection on the plants was present in the fall of 1918. However, owing to the extensive defoliation, very little overwintering of viable bacteria occurred in the old canker spots on the leaves, so most of overwintering must have taken place in the cankers on the twigs and stems of the grapefruit, Satsuma, and trifoliate orange plants.

As deciduous Citrus plants such as the trifoliate orange shed their leaves each fall, infection in the spring must come only from the viable spots on the twigs or from outside sources. In Plate 2, D, is shown the early spring development of active canker tissue immediately adjacent to and surrounding the typical cankers formed on the last growth of the preceding year. The darker area represents the oily, unruptured viable spots. The lighter zones surrounding them are the result of the spring activities of the organism. The lighter areas become evident soon after growth of the plants begins. These cankers develop rapidly and soon rupture. Between 50 and 75 per cent of the cankers of this type are viable in the spring.

Many of the trifoliate hybrids retain their foliage during the winter, so overwintering of the organism may occur in the spots formed in late summer as well as in the cankers which develop on the twigs.

[7] PELTIER, G. L., and NEAL, D. C. OVERWINTERING OF THE CITRUS-CANKER ORGANISM IN THE BARK TISSUE OF HARDY CITRUS HYBRIDS. Jour. Agr. Research 14: 523–524, illus. 1918.

Grapefruit and allied plants which react differently to environmental influences presented a somewhat different problem. As has been noted, twig cankers are produced late in the summer on angular wood. However, owing to the rather unstable dormancy of this plant, the organism can and does develop from time to time as the host responds to periods of favorable temperatures for growth. Consequently, the viable cankers do not go through the winter in unruptured condition. Such spots as these may extend down on the angular wood for some distance. During the winter 1919–20 these ruptured spots remained viable.

When defoliation is not complete, the organism is also able to overwinter in some of the spots on the leaves, and with the renewed activity in the spring many small new spots develop around the margin of the old cankers (pl. 1, D). These in turn serve as a source of infection for the new leaves which are developing. Another quite common source of infection present on grapefruit plants was found on or near the leaf scars; each spring many small viable cankers are found near the base of the leaf scars and these develop very much as those described on the twigs (pl. 3, C).

On Satsuma and plants of similar nature, the organism is overwintered, with but few exceptions, in the viable cankers on the leaves. The most common means by which the organism overwinters is in the spots formed late in summer on the last growth (pl. 1, A. B, C). The second means of overwintering is through delayed incubation, which has already been described (pl. 3, A). The same type of overwintering was again observed to be common during the season of 1920–21.

DISCUSSION

It has been shown that *Pseudomonas citri* can not live over the winter, except when in the host tissue, and then only in cankers developed the previous season. Even cankers formed during the early growing season are usually not viable. The cankers formed on the last growth during the season, either on leaves or twigs, are usually the ones which are the sources of the early spring infections. The activity of these old spots begins at the same time growth of the hosts starts, so by the time the new leaves are unfolding the cankers have ruptured and the disease spreads.

The method of overwintering through an extended incubation period which has been found to occur on Satsuma are merely late season infections which are formed on the last growth, but which do not develop sufficiently to produce a typical spot because of adverse environmental conditions. That they do spread somewhat internally during the dormant season can be shown by the fact that when they develop during the spring they seem to cover part of the leaves.

The citrus canker organism was not recovered from any of the cankers one year old or older, so it may safely be assumed that only some of the canker spots formed during the current year on leaves and angular wood on the trees are viable the following season.

SUMMARY

In southern Alabama, *Pseudomonas citri* Hasse is overwintered as follows:

(1) In old spots on the leaves which remain on the tree.

(2) In spots on the twigs formed near the end of the growing season, principally upon angular wood.

(3) By means of an extended or arrested incubation period of the organism on leaves and twigs of the last growth of the season. This method of overwintering is likely to occur only during seasons when frost is long delayed.

The number of viable spots which winter over is influenced by the severity of the winter. A severe winter causes almost complete shedding of the infected leaves, and kills back the angular wood.

The viability of an individual canker spot is more or less limited, and all spots on an infected tree do not serve to carry the organism through the winter.

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THE EFFECTIVENESS OF CERTAIN PARAFFIN DERIVATIVES IN ATTRACTING FLIES[1]

By WILLIAM C. COOK

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INTRODUCTION

A survey of the literature concerning substances used to attract insects furnishes very little information regarding the relative attractiveness of different members of the paraffin series. Richardson [2] found amyl alcohol more attractive to flies than ethyl alcohol when used at a concentration of 4 per cent. Speyer [3] tried various pure compounds of this series and concluded that the addition of successive CH_2 groups increased the attractiveness of the compound. He also states that amyl compounds are increasingly attractive in the order in which they are naturally formed during fermentation. Peterson [4] reported that the saturated alcohols were attractive to the adults of the onion maggot in the order iso-propyl, ethyl, butyl, amyl, methyl. He exposed his chemicals in a honey solution at about 1.5 per cent concentration. This concentration is near the optimum for iso-propyl alcohol, but is too weak for ethyl and too strong for butyl and amyl alcohols.

The experiments on which this paper is based were planned for the purpose of determining the relations, if any, between the physical properties of the various compounds and their attractiveness to flies. The results will be presented under four headings: (1) The time factor in bait experiments; (2) the relations of physical properties to the dilution most attractive to flies; (3) the relative attractiveness of the paraffin alcohols and esters; and (4) the relative attractiveness of normal and branched-chain compounds.

EXPERIMENTAL TECHNIQUE

These experiments were conducted at Bozeman in the summer of 1923. Flies were abundant, and a total of 50,000 were captured. About 50 small wire balloon-type flytraps were used, and these were kept in one location during the entire summer. Since preliminary experiments had indicated that the traps could be run quite close together without influencing the results, about four or five were placed 18 inches apart in a circle, and several circles were placed on a line. Figure 1 shows the arrangement of one-half of the series during one experiment. When check traps were used they were placed between the circles, which were then moved farther apart.

The standard or basic bait was 25 c. c. of 10 per cent cane molasses solution. A sufficient quantity of the chemical was added to this to give the desired concentration. Very dilute solutions of a chemical

[1] Received for publication Mar. 14, 1925; issued February, 1926.

[2] RICHARDSON, C. H. THE RESPONSE OF THE HOUSE-FLY TO CERTAIN FOODS AND THEIR FERMENTATION PRODUCTS. Jour. Econ. Ent. 10: 102-109. 1917.

[3] SPEYER, E. R. NOTES ON CHEMOTROPISM IN THE HOUSE-FLY. Ann. Appl. Biol. 7: 124-140. 1920.

[4] PETERSON, A. SOME CHEMICALS ATTRACTIVE TO ADULTS OF THE ONION MAGGOT (HYLEMYIA ANTIQUA MEIG.) AND THE SEED-CORN MAGGOT (HYLEMYIA CILICRURA ROND.). Jour. Econ. Ent. 17: 87-94. 1924.

were obtained by successive dilutions of a concentrated solution. After the chemical was added the bait solution was absorbed on sufficient bran to make a wet mash. This retarded the evaporation of the solution and greatly facilitated handling.

The chemicals used in the experiments, together with their formulae and boiling points, are given in Table I.

TABLE I.—*Chemicals used in experimental work*

Name and grade	Boiling point (° C.)	Formula
Ethyl alcohol, absolute	78.4	C_2H_5OH.
Ethyl acetate, practical	71 to 74	$CH_3COOC_2H_5$.
Ethyl propionate, technical	85 to 102	$C_2H_5COOC_2H_5$.
Ethyl butyrate, technical	108 to 122	$C_3H_7COOC_2H_5$.
Ethyl n-valerate, c. p	144 to 144.5	$C_4H_9COOC_2H_5$.
Iso-propyl alcohol, 90 to 95 per cent	80 to 81	C_3H_7OH.
Iso-propyl acetate, c. p	87 to 88	$CH_3COOC_3H_7$.
N-propyl n-propionate, c. p	122 to 124.5	$C_2H_5COOC_3H_7$.
N-propyl n-butyrate, c. p	142 to 144.5	$C_3H_7COOC_3H_7$.
N-propyl iso-valerate, technical	150 to 160	$C_4H_9COOC_3H_7$.
Iso-butyl alcohol, c. p	106 to 108	C_4H_9OH.
N-butyl acetate, c. p	124 to 126	$CH_3COOC_4H_9$.
N-butyl propionate, c. p	142 to 144	$C_2H_5COOC_4H_9$.
N-butyl butyrate, technical	155 to 167	$C_3H_7COOC_4H_9$.
N-butyl n-valerate, c. p	183 to 185	$C_4H_9COOC_4H_9$.
Iso-amyl alcohol, c. p	130 to 132	$C_5H_{11}OH$.
Iso-amyl acetate, technical	110 to 140	$CH_3COOC_5H_{11}$.
Iso-amyl propionate, c. p	159 to 161	$C_2H_5COOC_5H_{11}$.
Iso-amyl butyrate, technical	159 to 179	$C_3H_7COOC_5H_{11}$.
Iso-amyl valerate, technical	65 to 80/8 mm	$C_4H_9COOC_5H_{11}$.

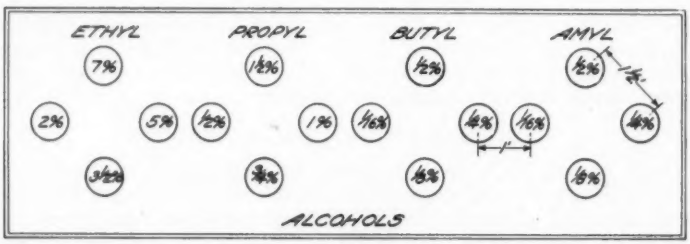

FIG. 1.—Arrangement of part of the trap series in the alcohol experiments

THE TIME FACTOR IN BAIT EXPERIMENTS

In determining the relative attractiveness of two substances which differ in boiling point and probably also in volatility, it is impossible to draw conclusions without considering the time factor. The more volatile compound may be more attractive at first, but will evaporate more quickly, whereas the less volatile compound will remain attractive for a longer time. In order to test this point several experiments were made, in each of which the most attractive concentrations of several compounds were exposed together until only a few flies were attracted. The flies were collected and counted every 24 hours. The combined results of three experiments, each run for 72 hours, are given in Table II.

TABLE II.—*Influence of length of exposure upon relative attractiveness of paraffin alcohols to flies*

Exposure period	Total number of flies captured in each period by use of—			
	Ethyl alcohol (7 per cent)	Propyl alcohol (1 per cent)	Butyl alcohol (3/4 per cent)	Amyl alcohol (1/4 per cent)
First 24 hours	328	232	108	74
Second 24 hours	168	209	63	92
Third 24 hours	144	222	223	146
Total (72 hours)	640	663	394	312

It will be noted that ethyl alcohol caught the largest number of flies during the first period; iso-propyl alcohol during the second period; and butyl alcohol during the third period. When the captures for the three periods are combined, iso-propyl alcohol is seen to have captured the greatest number.

If it had been possible to continue the experiments for a longer period, the higher alcohols would have caught still more flies. A single experiment, not included in the above table, showed that at the end of five days (120 hours) iso-amyl alcohol had caught a much larger number of flies than ethyl alcohol.

It is evident that a standard exposure period must be used if the results of different experiments are to be comparable. For that reason all the remaining experiments cited in this paper were run for a uniform exposure period of 24 hours.

RELATION OF PHYSICAL PROPERTIES TO THE DILUTION MOST ATTRACTIVE TO FLIES

METHODS

For these experiments the traps were arranged as shown in Figure 1. Several dilutions of a single compound were placed in a circle, and usually four such circles were set out, each containing a different compound. The whole set was duplicated, so that two traps of each dilution of each compound were exposed at once. No control traps were used, since the object of the experiments was to find the most attractive dilution of each chemical, and not the relative attractiveness of the various chemicals.

RESULTS

The work was started on an empirical basis with the four alcohols, and the detailed results for ethyl alcohol are given in Table III.

TABLE III.—*Flies caught on various dilutions of ethyl alcohol*

Per cent solution	Experiment No.								Total
	1	2	12	13	14	23	24	25	
10	[a] 12	16							28
7		41	8	15	30		235	143	472
6			5	10	7				22
5	19	31	7	11	20		273	93	454
4			5	14	21				40
3						103			103
2.5		28							28
2						143	208	99	450
1.5						109			109
1						117	172	161	450
Total	31	116	25	50	78	472	888	496	2,156

[a] Each number represents the total flies caught in two traps in 24 hours.

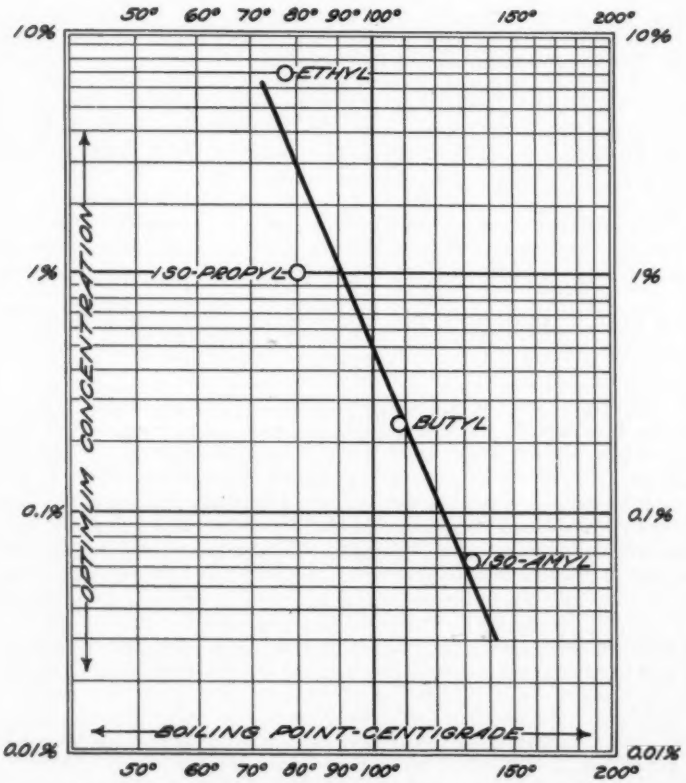

FIG. 2.—Relation of optimum concentrations of alcohols to their boiling points

DISCUSSION

An examination of Table III shows that the 7 per cent ethyl alcohol was the most attractive dilution. The 5 per cent alcohol caught nearly as many flies, but the 7 per cent ranked higher in five of the six experiments in which the two dilutions were exposed together. It is out of the question to present detailed results for all of the compounds in this paper. The complete data are in the files of the entomology department, Montana Experiment Station. The following dilutions of alcohols were found most attractive to flies:

Ethyl alcohol, 7 per cent; boiling point, 78° C.
Iso-propyl alcohol, 1 per cent; boiling point, 80° to 81° C.
N-butyl alcohol, ¼ per cent; boiling point, 106° to 108° C.
Iso-amyl alcohol, $\frac{1}{16}$ per cent; boiling point, 130° to 132° C.

If the logarithms of these concentrations be plotted against the logarithms of the boiling points, the points so found will lie nearly on a straight line (fig. 2).

Using this relation as a working basis, the other compounds were tested in concentrations lying above and below the intersections of their boiling points with this line. In each case the optimum concentration lay fairly close to the line.

The most attractive concentration of each of the 20 compounds used, together with some of the physical constants of each compound, is given in Table IV. The writer is indebted to Jean Timmerman, of the University of Brussels, Belgium, for data on the freezing points of these compounds.

TABLE IV.—*Most attractive dilutions and some physical constants of the paraffin compounds used*

Compound	Molecular weight	Formula	Boiling point (° C.)	Freezing point (° C.)	Most attractive strength
					Per cent
Ethyl alcohol	46	C_2H_5OH	78.4	−114	7.000000
Propyl alcohol (iso)	60	C_3H_7OH	80 to 81	−127	1.000000
N-butyl alcohol	74	C_4H_9OH	116 to 118	−89.8	.250000
Iso-amyl alcohol	88	$C_5H_{11}OH$	130 to 132		.062500
Ethyl acetate	88	$CH_3COOC_2H_5$	71 to 74	−82.4	1.000000
Iso-propyl acetate	102	$CH_3COOC_3H_7$	87 to 88	−73.4	.500000
N-butyl acetate	116	$CH_3COOC_4H_9$	124 to 126	−76.8	.062500
Iso-amyl acetate	130	$CH_3COOC_5H_{11}$	138 to 140		.015625
Ethyl propionate	102	$C_2H_5COOC_2H_5$	85 to 102	−73.9	.250000
N-propyl n-propionate	116	$C_2H_5COOC_3H_7$	122 to 124.5	−75.9	.200000
N-butyl propionate	130	$C_2H_5COOC_4H_9$	142 to 144	−89.2	.100000
Iso-amyl propionate	144	$C_2H_5COOC_5H_{11}$	159 to 161		.025000
Ethyl butyrate	116	$C_3H_7COOC_2H_5$	119 to 121	−93.3	.400000
N-propyl n-butyrate	130	$C_3H_7COOC_3H_7$	142 to 145	−95.2	.037500
N-butyl butyrate	144	$C_3H_7COOC_4H_9$	163 to 166	−97.5	.020000
Iso-amyl butyrate	158	$C_3H_7COOC_5H_{11}$	159 to 179		.007500
Ethyl n-valerate	130	$C_4H_9COOC_2H_5$	144 to 144.5	−89.9	.100000
N-propyl iso-valerate	144	$C_4H_9COOC_3H_7$	150 to 160		.025000
N-butyl n-valerate	158	$C_4H_9COOC_4H_9$	183 to 185		.005000
Iso-amyl valerate	172	$C_4H_9COOC_5H_{11}$	194		.002500

The relation between the boiling points of these paraffin compounds and their most attractive dilutions is shown in Figure 3. This graph is plotted on logarithmic paper on which the horizontal scale is about six times the vertical scale. The figures given for each compound represent the number of flies per trap per day caught in all experiments with each concentration. In this way it is possi-

ble to show on one chart the concentrations used and the relation between boiling point and most attractive concentration.

There are some noticeable irregularities in the positions of the points with regard to the line, but the fit is as close as can be expected, for there is necessarily a high experimental error in this work.

Within the limits of error, it may be stated that the most attractive concentration of an alcohol or ester of the paraffin series bears a close relation to the boiling point of the compound. The concentration decreases rapidly with an increase in the boiling point, and when plotted logarithmically a straight line is obtained. The approximate formula of this line is

$$\text{Log. opt. sol.} = 13.78 - 7.1 \log. \text{B. P.}$$

showing that the attractive amount decreases about seven times as rapidly as the log. boiling point increases.

THE RELATIVE ATTRACTIVENESS OF THE PARAFFIN ALCOHOLS AND ESTERS TO FLIES

METHODS

The only important change in these experiments was the introduction between the circles of control traps, baited with 10 per cent molasses absorbed on bran. This afforded a basis for comparing the various experiments.

RESULTS

Three sets of experiments were made. In the first set of three experiments all of the ethyl and propyl compounds were tested at their most attractive concentrations. Four traps of each compound were used in each experiment. In the second set of two experiments four traps of each of the butyl and amyl compounds were used. In the third set of nine experiments two traps of each of the entire series of compounds were used. The results of these three series of tests are given in Tables V, VI, and VII.

TABLE V.—*Number of flies caught on ethyl and propyl compounds*

	Alcohol	Acetate	Propionate	Butyrate	Valerate	Total
Ethyl	2,704	2,564	1,570	967	730	8,535
Propyl	1,403	[a] 1,272	1,844	1,233	373	6,125
		[a] (1,798)				[a] (6,651)
Total	4,107	3,836	3,414	2,200	1,103	14,660
		[a] (4,362)				[a] (15,186)

Four control traps caught 773 flies.

[a] Iso-propyl acetate was used in only two experiments. The figures in parentheses indicate an interpolated total based on the relative catch in experiments in which it was used.

TABLE VI.—*Number of flies caught on butyl and amyl compounds*

	Alcohol	Acetate	Propionate	Butyrate	Valerate	Total
Butyl	444	441	205	218	355	1,663
Amyl	286	563	333	205	70	1,457
Total	730	1,004	538	423	425	3,120

Four control traps caught 233 flies.

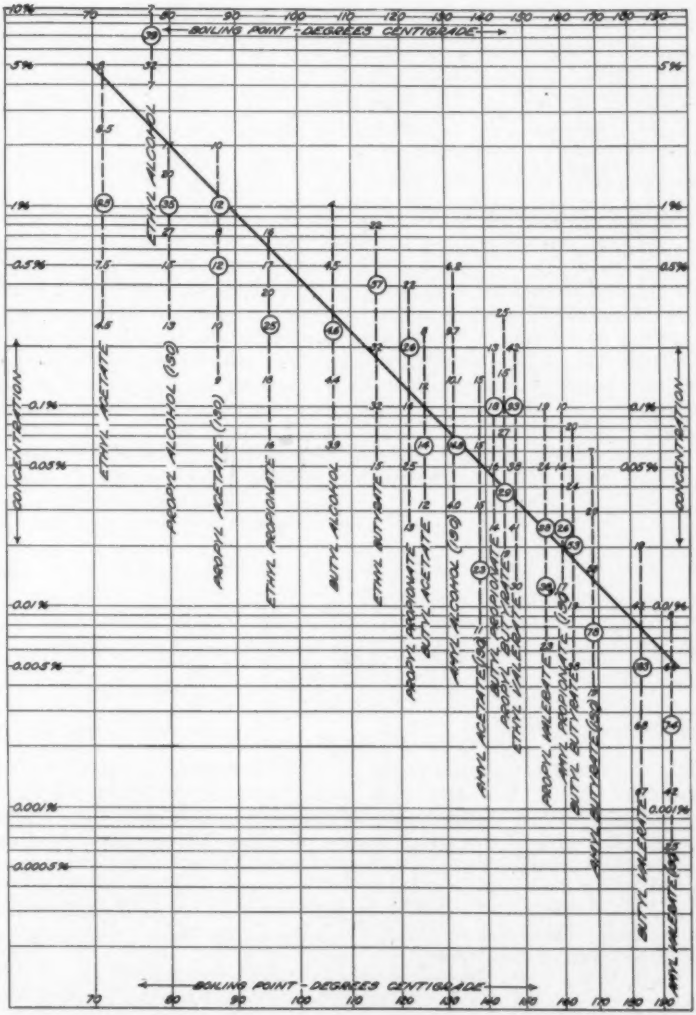

FIG. 3.—Relation between boiling points and optimum concentrations of paraffin compound. Small figures indicate flies per trap per day for each concentration tested. A circle is drawn around the figure for the most attractive concentration

TABLE VII.—*Number of flies caught on 20 paraffin alcohols and esters*

	Alcohol	Acetate	Propionate	Butyrate	Valerate	Total
Ethyl	858	1,202	174	123	226	2,583
Propyl	394	[a] 272 [a] (672)	340	246	49	1,301 [a] (1,701)
Butyl	148	460	446	130	125	1,309
Amyl	177	571	282	166	66	1,262
Total	1,577	2,505 [a] (2,905)	1,242	665	466	6,455 [a] (6,855)

Two control traps caught 165 flies.
[a] Iso-propyl acetate was used only in four experiments. Figures in parentheses indicate calculated values for nine experiments obtained by interpolation.

The data in Tables V, VI, and VII have been carefully studied and plotted. In order that the results of the first two series might be comparable, it was necessary to multiply the figures in Table VI by 3, since the control traps in the first series caught about three times as many flies as those in the second series. The figures in Table VI, multiplied by 3, were added to Tables V and VII. Since the combined results shown in Table VIII differ but slightly from those of the third series (Table VII), they are used as the basis of discussion.

TABLE VIII.—*Combined results of 14 experiments made to determine the relative attractiveness of paraffin compounds to flies*

	Number of flies caught on—					Total
	Alcohol	Acetate	Propionate	Butyrate	Valerate	
Ethyl	3,562	3,766	1,744	1,090	956	11,118
Propyl	1,797	2,470	2,184	1,479	422	8,352
Butyl	1,480	1,783	1,061	784	1,190	6,298
Amyl	1,035	2,260	1,281	781	276	5,633
Total	7,874	10,279	6,270	4,134	2,844	31,401

Control traps caught 1,637 flies.

The only notable difference in the relative position of the compounds in Tables VII and VIII is in the case of butyl valerate. This difference is doubtless due to experimental error. One single trap out of eight in the experiments of Table VI caught 129 flies, whereas the average for the other seven was 32 flies. This difference, multiplied by 3 in forming Table VIII, would account for 291 flies and reduce the total in that table to 899 flies, which would account for the one large discrepancy found in discussing the relative attractiveness of isomeric compounds.

DISCUSSION

If Table VIII is studied in detail several points of interest will be noted. First, there is, in general, a decrease in attractiveness with an increase in boiling point. This is shown by the figures in both columns of totals, and is well illustrated by the alcohols.

The esters constitute a separate group, and will repay further study. As they are located in the table each one differs from its neighbor

horizontally or vertically by one CH_2 group. The members of each vertical column differ by one CH_2 group on the alcohol side of the formula, whereas the members of a horizontal row differ by one CH_2 group on the acid side of the formula. Totaling the esters horizontally we get the following results:

Ethyl esters	7,566
Propyl esters	6,555
Butyl esters	4,818
Amyl esters	4,598

It is seen that the addition of CH_2 groups to the alcohol side of the formula has a slight effect upon the attractiveness.

Totaling the figures for the esters vertically, we get the following results:

Acetates	10,279
Propionates	6,270
Butyrates	4,134
Valerates	2,844

Here also there is a larger drop from acetates to propionates than is found higher up, but there is a considerable difference between the figures in each case, showing that the addition of a CH_2 group to the acid side of the radical has a relatively large effect upon the attractiveness of the compound to flies.

This is also shown in another way in Table VIII. Omitting the alcohols, the figures in each row decrease regularly from left to right. The one exception, butyl valerate, is probably due to experimental error, as shown above.

Several sets of esters in this group are isomeric; that is, they have the same empirical formula, differing only in the structure of the molecule. One set is that of ethyl propionate ($C_2H_5COOC_2H_5$) and propyl acetate ($CH_3COOC_3H_7$), the empirical formula for each of which is $C_5H_{10}O_2$. Sorting out the five such sets from Table VIII, we obtain the following results:

A	Ethyl propionate	1,744
	Propyl acetate	2,470
B	Ethyl butyrate	1,090
	Propyl propionate	2,184
	Butyl acetate	1,783
C	Ethyl valerate	956
	Propyl butyrate	1,479
	Butyl propionate	1,061
	Amyl acetate	2,260
D	Propyl valerate	422
	Butyl butyrate	784
	Amyl propionate	1,281
E	Butyl valerate	1,190
	Amyl butyrate	781

There are several irregularities in the table, but in general that member having the lowest acid radical (the lowest of each set as tabulated) is most attractive.

Finally, it will be noted that of 20 compounds studied only 8 were more attractive than the control traps. The remaining compounds were all more or less repellent in action.

The figures given in Table VIII are shown plotted on a logarithmic scale against the boiling points of the compounds in Figure 4. This shows in a more prominent manner the features found in the table.

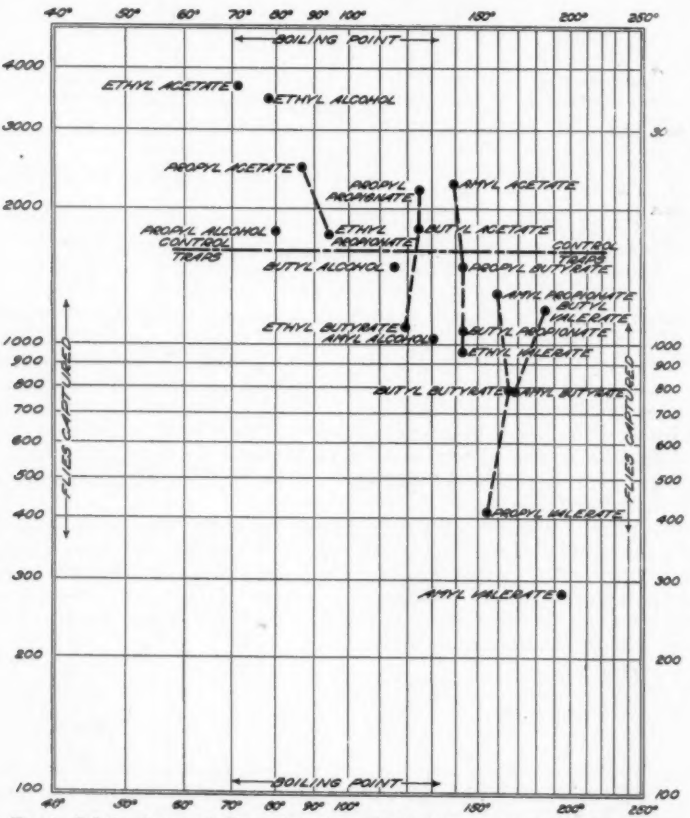

FIG. 4.—Relation between boiling points of paraffin alcohols and esters and their relative attractiveness to flies

RELATIVE ATTRACTIVENESS OF NORMAL AND BRANCHED-CHAIN COMPOUNDS

The final point covered in this investigation is the relative attractiveness of normal and branched-chain compounds. Such compounds as n-propyl alcohol and iso-propyl alcohol are isomeric, having the empirical formula C_3H_7OH. They differ in the arrangement of the atoms in the molecule. Normal propyl alcohol has the structural formula

$$\begin{array}{l} H\ H\ H \\ H-C-C-C-OH, \text{ or } CH_3CH_2CH_2OH, \\ H\ H\ H \end{array}$$

whereas iso-propyl alcohol has the structural formula

$$\begin{array}{l} \quad H \\ H-C-H \\ \quad \vdots \\ H-C-OH, \text{ or } (CH_3)_2\ CHOH. \\ \quad \vdots \\ H-C-H \\ \quad H \end{array}$$

The normal compound, as supplied by Eastman, boils at 96° to 98° C., whereas the iso compound boils at 81.5° to 82.1° C., about 15° lower.

RESULTS

Five pairs of such isomeres were studied, four traps of each chemical being exposed to flies at their optimum concentrations for three experiments. The results are given in Table IX.

TABLE IX.—*Relative attractiveness to flies of normal and iso compounds*

Compound	Formula	Boiling point (° C)	Number of flies captured	Ratio (normal=1)
Propyl alcohol:				
Normal	$CH_3CH_2CH_2OH$	96 to 98	243	1
Iso	$(CH_3)_2CHOH$	81.5 to 82.1	260	1.07
Butyl alcohol:				
Normal	$CH_3CH_2CH_2CH_2OH$	114 to 118	61	1
Iso	$(CH_3)_2CH.CH_2OH$	106 to 108	80	1.31
Amyl alcohol:				
Normal	$CH_3(CH_2)_3CH_2OH$	136 to 139	15	1
Iso	$(CH_3)_2CH.CH_2CH_2OH$	130 to 132	48	3.20
Propyl acetate:				
Normal	$CH_3COOCH_2CH_2CH_3$	99 to 102	98	1
Iso	$CH_3COOCH(CH_3)_2$	87 to 88	287	2.93
Butylpropionate:				
Normal	$CH_3CH_2COO(CH_2)_3CH_3$	142 to 144	21	1
Iso	$CH_3CH_2COOCH_2CH(CH_3)_2$	136 to 138	79	3.76

DISCUSSION

In every case the iso compound caught a larger number of flies than the corresponding normal compound. It would be expected that the iso compounds would be slightly more attractive, since their boiling points are lower, but the difference in attractiveness is not proportional to the difference in the boiling point. For example, the two propyl alcohols have the widest difference in boiling point of any pair in the tests, about 15° C., and yet they are the most nearly equal in attractiveness, whereas the two butyl propionates differ by but 6° in boiling point, but the iso compound is more than three times as attractive as the normal compound. The number of flies caught in these experiments is rather small to use in drawing definite conclusions, but it is evident that the iso compounds are more attractive than the normal compounds, and that the difference in attractiveness increases as the boiling points of the compounds increase.

SUMMARY

The experiments herein reported, in which over 50,000 flies were captured, have demonstrated the following points regarding the attraction of flies by organic chemicals of the paraffin series:

(1) There is a definite optimum concentration for each compound studied. This concentration is related to the boiling point of the compound, and becomes smaller as the boiling point rises.

(2) The relative attractiveness of paraffin alcohols and esters is related to the boiling points of the compounds. As the boiling point becomes higher the attractiveness decreases. This relation is somewhat obscured by two other relations: (*a*) The addition of a CH_2 group to the acid radical reduces the attractiveness much more than the addition of a similar group to the alcohol radical. (*b*) This is a corollary of *a*. Of a given set of isomeric compounds, that one is generally most attractive which has the lowest acid radical.

(3) Iso or branched-chain compounds are relatively more attractive than their normal isomeres. The difference in attractiveness increases as the boiling point increases.

THE PRACTICABILITY OF QUANTITATIVE TOXICOLOGICAL INVESTIGATIONS ON MANDIBULATE INSECTS [1]

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INTRODUCTION

The effects of stomach-poison insecticides have not yet received attention from a deliberately quantitative point of view. In this paper methods developed for administering poison solutions to mandibulate insects are described, and results obtained in experiments on the tent caterpillar (*Malacosoma americanum*) and the yellow-necked caterpillar (*Datana ministra*) are given.

METHODS

To make possible a quantitative toxicology of stomach-poison insecticides it is necessary to have dependable methods for quickly introducing known weights and volumes of poisons into the bodies of individual insects. Two methods are possible, namely, feeding by mouth and subintegumental injection. This paper deals with the administration of arsenicals by mouth. Poisons so taken are accepted only in conjunction with food, water, or both. They may be given in solid form, in suspension in water, or in aqueous solution.

Relatively insoluble commercial arsenicals must be offered to mandibulate insects in solid form or in suspension in water with the food of the insect. In the case of phytophagous insects the most obvious method of administration is to deposit on the leaf surface uniform drops of a suspension of the poison, allow these drops to dry, and then permit the insect to consume the dried drops with the leaf tissue. This method was tried on a small scale by Cook and McIndoo (*2, p. 46*).[2] Many difficulties are to be expected in the course of the experiments. (1) One can not be certain of the uniformity of drops from a pipette containing a quickly settling arsenical suspension; (2) the probability is small that an insect will consume within a reasonably short time the total leaf area occupied by one or more dried drops; (3) even if entire drops are apparently consumed, it is likely that some of the dried arsenical has flaked off before the advancing mandibles and become lost; (4) even if the dried drops were perfectly consumed, the actual time for the consumption of a lethal dose is so long and variable that the period of survival, that is, the length of time that the insect lives after consuming the poison, is difficult to estimate; and (5) continuous observation of each feeding insect is necessary, so that very few can be fed. Perhaps these considerations led Cook

[1] Received for publication Feb. 21, 1925; issued February, 1926: Paper No. 227 of the Journal series, New Jersey Agricultural Experiment Station, Department of Entomology. The work herein reported was carried out under the direction of T. J. Headlee. To him and to other members of the Rutgers faculty the writer is indebted for suggestions and criticisms.

[2] Reference is made by number (italic) to "Literature cited," p. 365.

and McIndoo (*2, p. 29*) to doubt the possibility of feeding known quantities of commercial insecticides to individual insects. It might be possible to overcome the foregoing difficulties by spraying leaves uniformly, allowing them to dry, and then permitting the insects under observation to feed upon them for known periods of time. The doses could be found later by analysis of the insects and their excreta (*8, p. 41*).

The toxic element or radical of practically every insoluble stomach-poison insecticide can be obtained in soluble form in a different chemical combination. Since the toxic element or radical in an insoluble compound must be made soluble in the insect intestine before it can exert its characteristic effect (*12*), it seems advantageous for the sake of understanding the mechanism of action of the toxic principle to administer it in solution.

Probably Price (*10*) was the first to feed known quantities of a poison solution to individual insects. He allowed honeybees to feed from a drop of a solution of sugar and sodium arsenate borne on a piece of wax foundation, the quantity of poison consumed being determined by the difference in weight of the wax foundation before and after the bee had fed. So far as the writer knows, mandibulate insects, against which stomach poisons are applied, have never been used for the quantitative feeding of poison solutions. In fact it is probably not generally realized that mandibulate insects, other than hymenopterous forms, can and do drink, for references to their ability to do so are scarce. Glaser (*4, p. 118*) introduced the virus of the gypsy-moth wilt disease into gypsy-moth caterpillars by allowing them to drink drops of water containing the virus from the tip of a pipette. Crozier (*3, p. 241*) observed the drinking of strychnine sulphate solution by caterpillars of *Protoparce celeus*, and other forms. In 1923 the writer noticed that the caterpillars of *Philosamia cynthia*, *Datana ministra*, *D. perspicua*, and *Malacosoma americanum*, which were feeding in the field drank completely drops of water placed in their feeding path. These caterpillars will also drink arsenical solutions, provided the solutions are neither too acid nor too alkaline. A feeding method based on this fact is described below as it was used on 19 yellow-necked caterpillars of *D. ministra* in the last instar.

A nearly neutral solution of arsenious oxide (As_2O_3), containing 3.75 gms. of arsenic per liter was employed (*13, p. 651*). A weighing burette made from a 100 c. c. separatory funnel, the stem of which was cut short and drawn to a fine tip, was used to measure out drops of the solution. During weighings the tip was covered by a short piece of test tube to prevent loss of weight by evaporation.

At 8.30 a. m. on August 25, 1923, 36 yellow-necked caterpillars were taken from a single colony on a Crataegus tree, brought to the laboratory, and labeled by sticking with collodion a bit of paper bearing the number to the prothorax of each caterpillar. Each larva was then weighed to the nearest centigram, and transferred to small branches from the host tree which had been inserted in bottles containing water. It was not necessary to cage the branches, which were, therefore, immediately accessible to the investigator throughout the experiment. When the caterpillars had become accustomed to their new situation and had begun to consume the foliage the test tube was removed from the stem of the burette (weighed to 0.1 mg.) and stoppered. A drop was deposited on the leaf surface in the feed-

ing path of the caterpillar about 2 mm. from its head. The tip of the burette was immediately covered again by the test tube, and the burette was hung on the balance. The advancing caterpillar was then observed until one of the scythelike sweeps of the head brought the mouth parts in contact with the drop. At this point the ventrad sweep of the head stopped instantly, and with its head motionless the insect rapidly imbibed the drop. As soon as the drop had disappeared the feeding movements of the head were resumed, and the portion of the leaf surface which the drop had occupied was eaten, thus insuring the complete consumption of the dose. The caterpillar was then transferred to fresh foliage and the burette was weighed. The difference in weight from the preceding observation gave the weight of the drop, from which the dosage in terms of metallic arsenic was later calculated. The specific gravity of the solution was taken into consideration in making the calculation.

As soon as the weighing was completed, the procedure just described was repeated on another conveniently situated caterpillar, and in this manner 19 caterpillars, ranging in weight from 0.31 to 0.90 gm., were fed varying quantities of the arsenical solution. An average of four per hour were handled. Continuous work was not always possible, for the caterpillars feed intermittently, and the individuals of a colony usually feed at the same time. Moreover, not every attempt to administer the arsenical solution was successful. Occasionally a caterpillar, disturbed by the movement of the leaf when the drop was deposited, stopped feeding and withdrew. In that case the portion of the leaf bearing the drop was cut off. Every caterpillar whose mouth parts touched a drop drank it completely. No caterpillar fed again after taking the poison except one individual which had received the smallest dose of all. Twelve unpoisoned caterpillars served as controls; they were in good condition after the poisoned larvae had died.

The observations to determine the death points of the poisoned caterpillars extended to midnight, the whole experiment thus lasting 16 hours. The caterpillars were pronounced dead when they failed to move on being lightly pinched with forceps on the last abdominal segment. The survival periods so determined were probably correct within approximately 5 minutes. The temperature was not controlled in this or in the following experiment, but averaged about 26° C. in both.

RELATION OF THE SPEED OF TOXIC ACTION TO THE DOSAGE

It is customary in pharmacological work to calculate dosages to a unit weight of animal basis, since the dosage necessary to produce a given effect varies more or less directly with the weight. Moreover, this procedure makes it possible to compare results obtained on different animals. This practice has been followed successfully in all experiments of the writer.

To express graphically the relation between dose per unit weight of insect and its effect, another pharmacological method was adopted. Instead of plotting doses against survival periods, they were plotted against reciprocals of survival periods, which may be interpreted as representing speed of toxic action. If there is a direct relationship between dose and speed of toxic action it is represented by a straight line; if not, deviations from the straight line may be significant

(*9, 11, 12*). The data so plotted lie along the 45° line in Figure 1. Although a straight line seems to fit the points, it must not be concluded that there is necessarily a direct relation between dosage and speed of toxic action, even in the range covered by the observations, for the data are subject to a high probable error. For the analysis of such a curve each point should represent the mean of a sufficient number of observations to give a satisfactory probable error for each

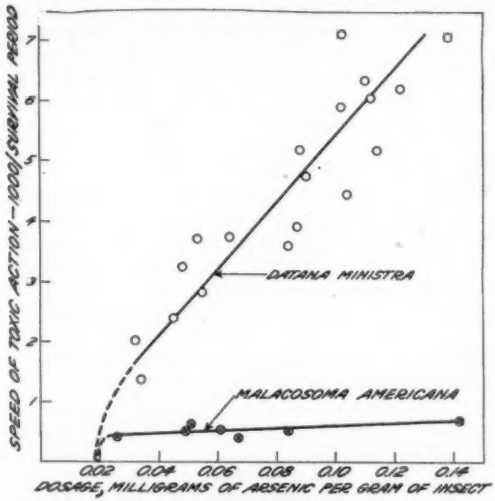
SPEED OF TOXIC ACTION—1,000/SURVIVAL PERIOD
DATANA MINISTRA
MALACOSOMA AMERICANA
0.02 0.04 0.06 0.08 0.10 0.12 0.14
DOSAGE, MILLIGRAMS OF ARSENIC PER GRAM OF INSECT

Fig. 1.—The relative susceptibility of two species of caterpillars to trivalent arsenic

mean. However, the true relation in this case can not be far from a direct one within a limited range of dosages. The data obtained from this experiment are given in Table I.

TABLE I.—*Data on the feeding of individual caterpillars of Datana ministra, with known doses of a neutral arsenite solution*

Insect No.	Weight of drop	Weight of insect	Weight of arsenic consumed per gram of insect	Survival period	$\frac{1,000}{\text{Survival period}}$
	Mg.	*Gm.*	*Mg.*	*Minutes*	
1	11.3	0.65	0.064	266	3.76
2	5.1	.40	.048	305	3.28
3	19.3	.63	.114	193	5.81
4	15.6	.48	.122	161	6.21
5	10.9	.37	.112	165	6.06
6	7.7	.90	.032	495	2.02
7	10.0	.41	.090	210	4.76
8	13.5	.61	.084	276	3.62
9	11.7	.40	.110	157	6.37
10	13.9	.38	.138	141	7.09
11	9.3	.34	.102	169	5.92
12	13.5	.49	.104	224	4.46
13	10.4	.74	.053	268	3.73
14	8.5	.59	.055	353	2.83
15	4.3	.48	.034	724	1.38
16	9.9	.41	.087	254	3.94
17	9.3	.40	.088	192	5.21
18	9.7	.34	.102	144	7.14
19	10.9	.90	.045	417	2.40

RELATIVE TOXICITY OF TRIVALENT AND PENTAVALENT ARSENIC

In all careful experiments on the relative toxicity of trivalent and pentavalent arsenic, from those made on single plant cells (*1*) to those on mammals (*5*), trivalent arsenic has been found the more toxic. One would expect the same thing to be true in the case of insects, and it has been so assumed by some recent writers on insecticides (*6, p. 624*), but apparently only one series of laboratory experiments has been made to test the generalization for insects (*2, p. 36*). The results were not conclusive, probably because the insoluble compounds used varied in rate of solubility within the alimentary tracts of the insects.

By feeding slightly alkaline solutions of an arsenite and of an arsenate of sodium of equal arsenic concentration to individual tent caterpillars (*Malacosoma americana*) the writer has proved qualitatively that the foregoing generalization holds for a representative of the insect class.

Both the arsenite and arsenate solutions were prepared from equal weights of recrystallized and resublimed arsenious oxide. For the preparation of the aresenite solution, 2 gm. of the oxide were dissolved in 11 c. c. of 0.1 normal sodium hydroxide (NaOH), and the resulting solution was diluted to 100 c. c. For the preparation of the arsenate solution, 2 gm. of the oxide were oxidized on the water bath with concentrated nitric acid (HNO_3). The excess nitric acid was driven off at 140° C. (*7, p. 1458*). The resulting arsenic oxide (As_2O_5) was redissolved and neutralized by the calculated volume of 0.1 normal sodium hydroxide to give a solution of sodium hydrogen arsenate (Na_2HAsO_4), which was diluted to 100 c. c. Therefore both solutions had a concentration of 15.15 gm. of arsenic per liter. The first had a hydrogen-ion concentration (P_H) greater than 10, the second a P_H of 8.8. One might expect a buffer action of the intestinal juices to eliminate the difference in P_H of the two solutions. However that may be, the results are valid, for they have been checked several times in experiments to be described in a subsequent paper in which the P_H of the two solutions was the same.

The experiments on the tent caterpillar in the last instar were essentially like those described for the yellow-necked caterpillar. A few differences in treatment may be mentioned. The tent caterpillar can not be transferred by hand to the foliage of the experiment because it tends to crawl away after being handled. The wild-cherry foliage was, therefore, placed in a cage containing the larvae and allowed to remain there until a sufficient number had crawled out upon it, after which it was removed from the cage. Instead of labeling and weighing the caterpillars before beginning to poison them, each caterpillar was weighed immediately after it was poisoned and was confined with foliage in a numbered bottle. Preliminary experiments had shown that unpoisoned larvae would feed and grow in such confinement. No disease was observed among these caterpillars.

TABLE II.—*Data on feeding of individual caterpillars of Malacosoma americana with known doses of arsenite and arsenate solutions of equal arsenic concentrations*

Insect No.	Weight of drop	Weight of insect	Weight of arsenic consumed per gram of insect	Survival period[a]	10,000 / Survival period[a]
Arsenite:	*Mg.*	*Gm.*	*Mg.*	*Minutes*	
1	1.2	0.30	0.061	1,740	5.75
2	6.3	.51	.184	1,140	8.77
3	1.7	.49	.051	1,530	6.53
4	1.6	.30	.084	1,860	5.38
5	5.7	.59	.142	1,410	7.09
6	1.1	.61	.026	2,400	4.17
7	1.6	.50	.049	1,800	5.38
8	3.0	.71	.067	2,400	4.17
Arsenate:					
1	1.3	0.61	0.031	3,180	3.14
2	9.2	.86	.157	2,250	4.44
3	3.2	.26	.182	1,860	5.38
4	6.1	.46	.195	2,100	4.76
5	3.5	.42	.120	2,280	4.38
6	1.3	.37	.051	2,400	4.17
7	7.1	.66	.159	1,680	5.95

[a] Survival period: Interval of time between administration of dose and death of caterpillar.

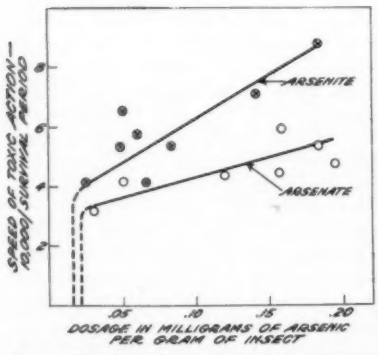

FIG. 2.—The relative toxicity of trivalent and pentavalent arsenic to the tent caterpillar (*Malacosoma americana*)

The data presented in Table II were secured in a continuous experiment in which the feeding was done in one afternoon. The survival periods are probably correct within approximately 30 minutes. In Figure 2 the distinct difference in location of the two curves represents a difference in toxicity of the trivalent and pentavalent arsenic, the former of which is seen to be the more toxic. A discussion of a numerical expression for the relative toxicity will not be attempted with the present data.

RELATIVE SUSCEPTIBILITY OF DIFFERENT SPECIES TO ARSENIC

It is of practical and theoretical importance to know whether or not different species react in the same way to doses of arsenic which are equivalent on a unit weight of insect basis. If the curves relating to dosage and speed of toxic action are practically the same in all cases, an important generalization will have been reached; if not, the differences in the curves, correlated with known anatomical and histological facts, may indicate the reason for the difference in susceptibility. In this section some evidence is presented to show that differences in susceptibility are to be expected.

The data of Price (*10, p. 6*) for bees which were fed known doses of sodium arsenate are not suitable for graphical comparison with the writer's data for pentavalent arsenic on tent caterpillars because the bees were not weighed. Nevertheless, if the dosages as given by

Price are transformed to an approximate gram weight of bee basis, the data are sufficient to show qualitatively that bees are more susceptible to pentavalent arsenic than tent caterpillars.

By reference to Figure 1, it is apparent that the yellow-necked caterpillar is more susceptible to trivalent arsenic than is the tent caterpillar. Temperature differences can not account for this difference in susceptibility, for both experiments were carried out at about 26 C. Moreover, the tent caterpillar drank more of the arsenic solution and ate for a longer time after drinking it than did the yellow-necked caterpillar. It seems improbable that the internal tissues of the tent caterpillar are as much more resistant to arsenic poisoning as the graphs would indicate, but rather that there is a chemical difference in the gut contents of the two forms, or a structural difference in the absorbing tissues of the alimentary tract which retards the rate of absorption of arsenic in the tent caterpillar.

It is interesting to observe, however, that the minimum lethal dose of both trivalent and pentavalent arsenic for the two species of caterpillars, represented by the probable points on the X-axis at which the curves intersect it, is of the same order of magnitude, and is in the neighborhood of 0.02 mg. of arsenic per gram of insect.

SUMMARY

The ability of certain mandibulate insects to imbibe completely drops of poison solutions has been utilized as a basis for the development of methods for the quantitative toxicological examination of soluble arsenical stomach-poison insecticides. By the use of these methods it has been found that trivalent arsenic is more toxic than pentavalent arsenic, that different species vary in susceptibility to arsenic poisoning, and that the minimum lethal dose of arsenic for the two species studied is about 0.02 mg. of arsenic per gram of insect.

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A GENETICAL STUDY OF THE FERTILITY OF THE LATERAL FLORETS OF THE BARLEY SPIKE[1]

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INTRODUCTION

Rather careful genetical studies have been made on certain phenotypical characters of the barley plant. Some of these characters are shown to be inherited in a simple Mendelian manner, while others present a more complex genetical composition. One of these is the "two and six rowed" character which is dependent upon the fertility of the side florets.

The head of the barley plant normally consists of three single-flowered spikelets at each node of the rachis. These groups of three spikelets are arranged alternately on the rachis, so that in appearance the head is composed of six vertical rows of spikelets extending from base to tip. In all varieties of barley the central floret develops a normal kernel, and, with the exception of the awnless sorts, bears either an awn or a hood on the outer flowering glume. With regard to the lateral florets, four degrees of fertility exist: (1) They may develop normal kernels, in which case the spike is six-rowed. Varieties of this type belong to the species *Hordeum vulgare*. The lateral floret glumes are usually awned or hooded similarly to the central ones. (2) The lateral florets may develop undersized kernels, and be awnless. The fertility in this case is intermediate between the two and six rowed types, that is, some of the lateral florets may be sterile. The intermediates all belong to the species *Hordeum intermedium*. (3) The lateral florets may be completely sterile (staminate only). This is the common two-rowed type known as *Hordeum distichon*. The side florets are awnless, and the tip of the outer glume is blunt or round. (4) Another two-rowed type has the sexual parts of the side florets entirely abortive, and the flowering glumes absent or reduced to mere traces. To this group is given the species name *Hordeum deficiens*. The above classification is according to Harlan (*4*).[2]

The inheritance of the two and six rowed character has been reported on by a number of investigators, with more or less disagreement in results. The earlier workers obtained results which indicated that the parental forms differ by a single pair of factors, which is possible only in cases where no homozygous intermediate type is obtained from a cross of two-rowed by six-rowed. Other workers have obtained such a homozygous intermediate class and have attempted to explain their results on the hypothesis that the parental forms differ in two or more factor pairs. It was for the

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[2] Reference is made by number (italic) to "Literature cited," p. 390.

purpose of obtaining more information on the inheritance of the fertility of the lateral florets that the work reported in this paper was undertaken.

PREVIOUS WORK

Investigators who have studied the inheritance of the "two and six rowed" character in barley are somewhat contradictory in their factorial interpretations. The earlier genetical studies led to the conclusion that these forms differ by a single factor. The F_2 ratios obtained were 1 homozygous six-rowed : 2 heterozygous intermediate : 1 homozygous two-rowed. The intermediate forms were heterozygous and segregated in the following generation. The best-known investigations in this connection are those carried on by Biffen (*1*, *2*) and Engledow (*3*).

Ubisch (*7*), in 1916, first suggested a two-factor hypothesis to account for fixed intermediate forms in the F_2 generation of crosses between six and two rowed barleys. The factor Z for two-rowed (zz = six-rowed) is associated with an intensifying factor W which is effective only in the presence of Z. The six-rowed forms are zzWW, zzWw, and zzww, and can not be distinguished, inter se, phenotypically. These make up 25 per cent of the progeny, which agrees with the results of previous workers. Seventy-five per cent of the progeny is made up of homozygous and heterozygous two-rowed and homozygous and heterozygous intermediate forms. The ZZWW and ZZww classes give only two-rowed and intermediate forms, respectively. A peculiarity arises in the F_2 phenotypic classification when the cross is between awned and hooded parents. The hooded plants show a 5 : 7 : 4 ratio for two-rowed, intermediate, and six-rowed forms, while the awned plants show the same classes in the ratio 3 : 9 : 4. Combining the two ratios on a 3 : 1 basis for hoods and awns, the F_2 ratio becomes 18 : 30 : 16, which approximates a 1 : 2 : 1 ratio obtained by previous investigators.

In 1920 Harlan and Hayes (*5*) suggested a two-factor hypothesis to explain the appearance of homozygous intermediate forms in the F_2 generation of a cross between six-rowed and two-rowed barleys. There are three six-rowed forms represented, as follows: AABB, homozygous six-rowed; AAbb, homozygous regressive six-rowed; and AABb, a form heterozygous for six-rowed and regressive six-rowed. These are indistinguishable phenotypically and make up 25 per cent of the total progeny. The homozygous intermediate class is aaBB, and the homozygous two-rowed class is aabb. The remaining combinations account for the various heterozygous classes obtained in the F_2 generation.

Hor in 1924 (*6*) has stated that the factors for *deficiens*, *distichon*, and *vulgare* types form a series of multiple allelomorphs, and that crossing any two will not produce the third. There is evidence presented by Ubisch (*8*) in 1923 which is contradictory to this assumption. From a cross between *deficiens* and six-rowed forms, two-rowed plants were produced in the ratio of 9 two-rowed, 3 six-rowed, 4 *deficiens*. Two factors, the writer assumes, differentiate the six-rowed and *deficiens* types; two dominant factors, ZD, produce the two-rowed type; D alone (with z) gives six-row; and Z or z with d gives *deficiens*.

MATERIALS AND METHODS

The following four types and varieties under each were used in the crosses made:

Six-rowed, *Hordeum vulgare:*
Manchuria, Oderbrucker, Featherston, Peruvian, *aethiops*, White Hull-less Himalaya, Mariout.
Intermediates, *Hordeum intermedium:*
Nudimortoni, nudihaxtoni, cornutum, nudihaxtoni nuditonsum, mortoni Nigritonsum, haxtoni intermedium.
Two-rowed, *Hordeum distichon:*
Palmella hypianthinum, McEwans Hull-less, Frankish, New Zealand, Red Barley, *primus, subcornutum.*
Deficiens, Hordeum deficiens:
Tridax, Two-rowed Hull-less, *deficiens, nudideficiens, steudelii.*

All the above-named sorts were from pure lines and have shown no segregation for fertility of the side florets since making the crosses.

The F_1 plants and those used for crossing were grown in the greenhouse. The F_2 and F_3 generations were grown in the field, in rows 5 feet long, and the seeds 2 to 3 inches apart. Complete F_2 and F_3 progenies were grown from three crosses. From the remaining crosses only complete F_2 generations were grown.

EXPERIMENTAL DATA

CROSS OF MANCHURIA × TRIDAX

Manchuria (*Hordeum vulgare*), which was used as the female parent, has the side florets fully fertile (six-rowed), while *tridax*, the male parent, is a two-rowed *deficiens* type with the side florets practically absent, and is classed by Harlan (*4*) as *Hordeum deficiens.*

Ten F_1 plants were obtained. These were all two-rowed, with the side florets slightly developed, but all sterile. The parental and F_1 types are shown in Figure 1.

F_2 Generation

The segregation of the F_2 generation is given in Table I. Of the 449 F_2 plants obtained, 113 were six-rowed and 336 were either *deficiens* or had the side florets very slightly developed, as in the F_1 (referred to as "two-rowed" in Tables I and II). This is a close approximation to a 1:3 ratio, the theoretical expectancy being 112.25 six-rowed and 336.75 two-rowed. The probable error based on a 1:3 ratio for 449 individuals is 6.19, and the deviation of 0. 75 is but 0.12 times the probable error. The probability that such a deviation may be obtained by random sampling is very great. It was impossible to distinguish definitely the heterozygous plants from the homozygous *deficines* plants. Of the 336 plants which were classed in F_2 as either homozygous *deficiens* or heterozygous two-rowed type, 56, or 16.7 per cent, were classed wrongly, as their behavior in the F_3 showed. In Figure 2 are shown the various F_2 forms produced.

FIG. 1.—Parental types and the F_1 of a cross of Manchuria × *tridax*

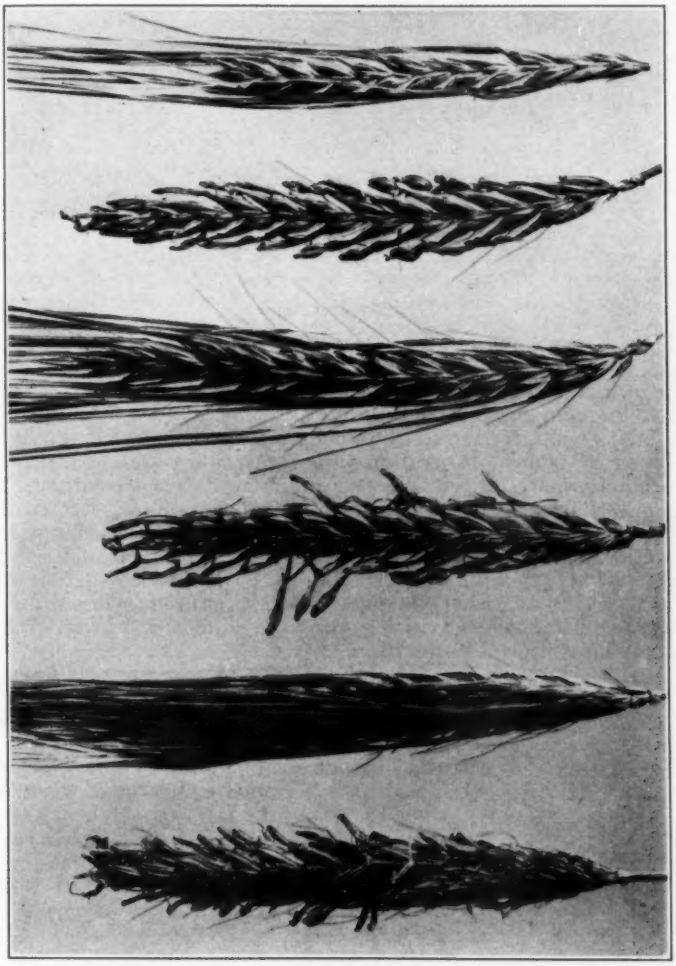

FIG. 2.—F_1 types obtained from a cross of Manchuria × *tridax*

TABLE I.—*Summary of the F_2 segregation of a cross of Manchuria × tridax*

	Number of F_2 plants	Six-rowed	Two-rowed
Observed	449	113	336
Calculated (1:3 ratio)		112.25	336.75
Deviation		0.75±6.19	

TABLE II.—*Classification of 370 F_2 segregates as determined by their F_3 progenies of a cross of Manchuria × tridax*

	Number of F_2 plants	Six-rowed	Heterozygous two-rowed	Two-rowed
Observed	370	92	186	92
Calculated (1:2:1 ratio)		92.5	185.0	92.5

$\chi^2 = 0.0108$. P = very large

F_3 GENERATION

Three hundred and seventy F_3 progenies containing 5 or more plants [3] were obtained from the F_2 plants. The F_3 results are summarized in Table II. Ninety-two progenies contained all six-rowed plants, therefore the F_2 plants producing these progenies must have been homozygous for the six-rowed character. Ninety-two F_2 plants produced all two-rowed plants of the *deficiens* type and must have been homozygous for the *deficiens* character. The remaining 186 progenies contained both six-rowed and two-rowed forms like the F_2. The plants producing these progenies were therefore heterozygous. The ratio 92 six-rowed : 186 heterozygous : 92 *deficiens*, closely approximates a 1:2:1 monohybrid ratio, the theoretical expectancy being 92.5 : 185.0 : 92.5. The value of χ^2 is extremely small, being equal to 0.0108, which indicates a close agreement between the observed and theoretical numbers.

DISCUSSION OF RESULTS

From the above data it is clear that the Manchuria and *tridax* strains used differ genetically by a single pair of allelomorphic factors for fertility of the side florets. These results do not agree with those reported by Ubisch (*8*) in which he obtained a two-rowed *distichon* form from a cross of six-rowed by *deficiens*. His hypothesis of a two-factor difference between six-rowed and *deficiens* assumes two genetically different *deficiens* types, one with the factor Z and one with z. When the one lacking Z is crossed with a six-rowed form, no homozygous two-rowed (*distichon*) forms can result.

[3] In order to avoid an error in classing some heterozygous progenies as homozygous dominants, all F_3 progenies of fewer than 5 plants of this and the following crosses were discarded. It is recognized that a "lower limit" of 5 individuals per progeny is entirely too small to be sure that an apparently homozygous dominant progeny is not in reality heterozygous. To obtain a 30:1 probability that a dominant class represents only dominants requires 12 individuals. However, in discarding all progenies of fewer than 12 plants the 1:2:1 ratio is decidedly disturbed. The homozygous recessive plants (six-rowed) produce more seeds per plant than the two-rowed plants, and their chances of producing progenies of 12 or more plants are greater than for the heterozygous or homozygous two-rowed dominants. Within certain limits, the larger the number of plants per progeny used as the limit in discarding progenies, the more the selection of the eliminated progenies will deviate from a random selection of the progenies from two and six rowed F_2 plants. For this reason the elimination of all progenies of fewer than 12 plants would not nearly so closely represent a random selection of recessives and dominants among the progenies eliminated as would a limit of 4 plants. The selection of the number 4, however, is purely arbitrary.

If the two-rowed *deficiens* character of the *tridax* parent is due to the factor Z for two-rowed, then the Manchuria strain must be zz and *tridax* ZZ. The intensifying factor W apparently is not concerned. On the other hand, if, for full fertility of the side florets, a third factor D is necessary, as suggested by Ubisch (*8*), and that its absence results in a *deficiens* type regardless of the Z factor (*deficiens* being zzdd or ZZdd), then the genetic constitution of the parents is as follows: Manchuria = zzWWDD and *tridax* = zzWWdd.

The presence of the modifying factor W has not been proved by these data. What effect the W factor might have on the character produced by the *deficiens* factor d is a matter for speculation. The *tridax deficiens* strain which is hooded differs from some of the awned *deficiens* strains in that the outer glumes of the side florets are fairly well developed and traces of the flowering glumes and rachilla are sometimes present. Whether or not this condition is due to the presence of the W factor can not be determined without more data.

CROSS OF ODERBRUCKER AND PALMELLA HYPIANTHINUM

Oderbrucker, the female parent, is a six-rowed fully awned type, *Hordeum vulgare*, practically the same as the Manchuria variety. The male parent, *palmella hypianthinum*, is the usual two-rowed type of barley, *Hordeum distichon;* the flowering glumes of the side florets are slightly developed but sterile and the central florets are awned.

Seven F_1 plants were obtained. The central florets were fully awned. The side florets were mostly fertile and bore short awns. The kernels produced by the side florets were of fair size although slightly smaller than the normal side-spikelet kernels of the six-rowed parent. The parental and F_1 forms are shown in Figure 3.

F_2 Generation

A summary of the F_2 results is given in Table III. The 378 F_2 plants obtained were classified as follows: 97 were six-rowed, side florets all fertile and fully awned; 188 plants had highly fertile side florets which were short awned or awn-pointed similar to the F_1; 51 had awnless side florets of low fertility, somewhat enlarged, and mostly sterile with the ends of the glumes round or blunt; and 42 were two-rowed like the male parent, with side florets all sterile (staminate). This approximates a 4 : 8 : 3 : 1 ratio, the expected frequencies being 94.5 : 189.0 : 70.9 : 23.6. The fit between the observed and calculated frequencies is fairly close, except in the last two classes, where there is an excess of two-rowed plants in the fourth class and a deficiency in the third class, due to errors in classification.

TABLE III.—*Summary of the F_2 segregation of a cross of Oderbrucker × palmella hypianthinum*

	Number of F_2 plants	Class 1, six-rowed	Class 2, highly fertile side florets with short awns	Class 3, enlarged awnless side florets, slightly fertile or nearly sterile	Class 4, two-rowed
Observed	378	97	188	51	42
Ratio per 16		4.1	8.0	2.1	1.8
Calculated (4 : 8 : 3 : 1 ratio)		94.5	189.0	70.9	23.6

$\chi^2 = 20.0031$ $P = 0.000170$

FIG. 3.—Parental types and the F_1 of a cross of Oderbrucker × *palmella hypianthinum*

The proportion of six-rowed plants to all others is 97:281 which is nearly a 1:3 ratio, the expected numbers being 94.5 and 283.5. This suggests that there is one essential factor difference between the six-rowed and two-rowed forms. However, the three distinct classes in which the 281 plants were classed indicate that there are at least two pairs of factors concerned, and that it is not a 1:2:1 segregation of six-rowed, intermediate, and two-rowed, as would be expected if the highly-fertile side florets with short awns and the intermediates with awnless side florets have the same genetical composition.

FACTORIAL HYPOTHESIS

The F_2 segregation may be explained by assuming the presence of the Z and W factors as suggested by Ubisch (7). Z is the essential factor for sterility of the side florets, W is an intensifying factor which accentuates the effect of Z. Both must be homozygous in the two-rowed type. The factors Ww or ww, when present, cause low fertility of the side florets, which are likewise awnless. With heterozygous Zz the fertility of the side florets is increased and the glumes have short awns or are awn-pointed, regardless of the presence or absence of W. The homozygous recessive zz is six-rowed, never two-rowed. The three six-rowed forms zzWW, zzWw, and zzww, are alike phenotypically. The fertility of the side florets is probably influenced also by environmental conditions. The genetic classes expected by the independent segregation of the Z and W factors are shown in Table IV.

This hypothesis agrees essentially with that proposed by Ubisch. By grouping all the intermediates together, Ubisch (7) obtained a ratio of 3:9:4 for two-rowed intermediates, and six-rowed in the case of the awned progeny. The ZZWw class above was classed by him as two-rowed and the ZZww class as intermediate. With the enlarged but sterile side-floret plants of class 2 included with the two-rowed plants, and the plants with slightly fertile side florets classed with the short-awned intermediates, the 4:8:4 ratio would probably be shifted to a 3:9:4 ratio. From the data at hand, the existence of the homozygous ZZww class is not demonstrated as will be shown by the F_3 data. However, if the complete F_3 generation were grown, such a class would probably be obtained.

TABLE IV.—*Genetic classes expected in the segregation of the F_2 progeny obtained from a cross of Oderbrucker × palmella hypianthinum*

Genotype	Per 16	Observed	Expected	Appearance of the phenotype
ZZWW 1	1	42	23.6	2-rowed; side florets staminate, sterile, and awnless.
ZZWw 2 ZZww 1	3	51	70.9	Side florets enlarged, awnless, and partially fertile or nearly sterile.
ZzWW 2 ZzWw 4 Zzww 2	8	188	189.0	Side florets mostly fertile, with awns or awn-pointed.
zzWW 1 zzWw 2 zzww 1	4	97	94.5	6-rowed; side florets fully fertile and awned.
Total		378	378.0	

F_3 Generation

Third-generation progenies were grown from three of the F_2 families, giving 111 F_3 families consisting of five or more plants each. The segregation of these three F_2 families is shown in Table V. The relative numbers in the various classes are practically the same as for the total F_2 population, with a similar discrepancy in classes 3 and 4.

TABLE V.—*Segregation of three F_2 families from a cross of Oderbrucker × palmella hypianthinum from which F_3 progenies were grown*

	Number of F_2 plants	Class 1, six-rowed	Class 2, highly fertile side florets with short awns	Class 3, enlarged, awnless side florets, slightly fertile or nearly sterile	Class 4, two-rowed
Observed	152	38	77	21	16
Ratio per 16		4.0	8.1	2.2	1.7
Calculated (4 : 8 : 3 : 1 ratio)		38.0	76.0	28.5	9.5

$\chi^2=6.434$ $P=0.094363$

A summary of the F_3 results showing the genotypic classes is given in Table VI. The 111 F_3 progenies were classified as follows: 5 contained all two-rowed plants; 14 segregated, giving two-rowed, partially fertile awnless side florets (intermediates), and heterozygous forms (presumably) with enlarged side florets which were practically all sterile; 21 segregated, giving six-rowed, highly fertile short-awned intermediates (heterozygous), and two-rowed forms (no awnless intermediates); 24 segregated, giving all expected classes; 14 segregated, giving six-rowed, highly fertile short-awned, and awnless intermediate forms, but no two-rowed forms; and 33 produced only six-rowed plants.

TABLE VI.—*Summary of the F_3 results of a cross of Oderbrucker × palmella hypianthinum*

Genotype	Per 16	Observed	Expected	F_3 segregation
ZZWW 1	1	5	6.9	All 2-rowed.
ZZWw 2	2	14	13.9	2-rowed, heterozygous, and low-fertility awnless side florets.
ZZww 1	1	0	6.9	All intermediate, awnless side florets, partially fertile.
ZzWW 2	2	21	13.9	6-rowed, highly fertile short-awned intermediates, and 2-rowed (no awnless intermediates).
ZzWw 4	4	24	27.7	All classes.
Zzww 2	2	14	13.9	6-rowed, highly fertile short-awned, and awnless intermediates (no 2-rowed).
zzWW 1 zzWw 2 zzww 1	4	33	27.7	All 6-rowed.
Total		111	110.9	

Of the 16 F_2 plants classed as two-rowed, 5 produced progenies containing only two-rowed plants, while 4 segregated as did the F_2 plants having enlarged awnless side florets and should have been classed with the F_2 plants in class 3. This difficulty in separating classes 3 and 4 in the F_2 classification accounts for the discrepancy in these two classes, Tables III and V.

In view of the small numbers obtained, as shown in Table VI, the observed fit the expected frequencies fairly closely, with two exceptions. There are no plants in the homozygous intermediate (ZZww) class, and there is an excess of plants segregating into six-rowed, highly fertile side florets bearing short awns, and two-rowed forms. The complete F_3 progeny would probably give a better fit in these two classes.

Discussion of Results

The necessity of at least a two-factor difference between the six and two rowed forms used in these crosses is shown by the above data. The fact that 25 per cent of the F_2 population were homozygous for the six-rowed character, that is a 3:1 segregation for two-rowed plus intermediates and six-rowed, indicates the operation of the essential factor Z for two-row, and is in agreement with the results of the cross of Manchuria × *tridax* and with practically all the results obtained in crossing two and six rowed forms. The variability of the segregates "not six-rowed" show in these, as in the results of many similar crosses, that a second factor is concerned. The second factor, which has been designated W, is not as effective as is the factor Z; that is, it is more or less overshadowed by Z, since the absence of Z results in complete fertility of the side florets regardless of the presence or absence of W. According to the factorial hypothesis suggested, the parents used contain the following factors: Oderbrucker = zzww(DD), and *Palmella hypianthinum* = ZZWW(DD). The factor D, which will be discussed later, may be present in both parents but is not concerned in the F_2 and F_3 segregation of this cross.

Cross of Two-Row Hull-Less × Nudimortoni

Two-row Hull-less, the female parent, is a two-rowed, awned, *deficiens* type, with the side florets practically absent. The internodes of the rachis are fairly long, resulting in a rather lax spike. The male parent, *nudimortoni*, is classed as an intermediate. The central florets are awned, the lateral florets are much enlarged and awnless, with the ends of the glumes round or blunt. The fertility of the side florets is rather low, many plants showing complete sterility when grown under greenhouse conditions. In the field the fertility is somewhat higher and appears to be influenced considerably by environmental conditions. The internodes of the rachis are fairly short, giving a short, dense spike. The segregation for spike density often made it very difficult to classify the plants with respect to type of side florets.

On each of the 13 F_1 plants obtained, the central florets were awned and the side florets were slightly developed but sterile, a condition intermediate between the parental types. The parental and F_1 types are shown in Figure 4.

F_2 Generation

The F_2 progeny contained 727 plants, which were classified in the following groups: (1) Side florets enlarged with one or more fertile; (2) side florets enlarged but all sterile; (3) side florets smaller than those of group 2 (intermediate between parental types, i. e., like the F_1); (4) side florets much reduced, and (5) *deficiens* type.

FIG. 4.—Parental types and the F_1 of a cross of two-row Hull-less × *nudimortoni*

As mentioned above, some of the F_2 plants were hard to classify because of the segregation of spike density. All gradations occurred between groups 3 and 5, inclusive, so that groups 3, 4, and 5 are probably not distinct genetic groups. The demarcation was fairly definite between groups 1 and 2 and between 2 and 3, although the difference between 1 and 2 may have been determined somewhat by environmental conditions. The F_2 progeny is summarized in Table VII.

TABLE VII.—*Summary of the F_2 segregation in a cross of two-row Hull-less × nudimortoni*

	Number of F_2 plants	Enlarged side florets		Side florets intermediate in size, all sterile, Group 3	Side florets much reduced, Group 4	Deficiens, Group 5
		One or more fertile, Group 1	None fertile, Group 2			
Observed	727	47	107	160	390	23
		154		573		
Ratio per 16		3.4		12.6		
Calculated (3:13 ratio)		136.3		590.7		
Deviation		17.7±7.1				

By combining groups 1 and 2 and groups 3, 4, and 5, the proportion of plants with enlarged side florets like the female parent (either all sterile or partially fertile), to all other plants is 154 : 573 which approximates a 3 : 13 ratio. The theoretical expectancy is 136.3 : 590.7, deviation is 17.7 ± 7.1.

FACTORIAL HYPOTHESIS

On the basis of the Z and W factor hypothesis, *nudimortoni* must contain ZZww and the Two-row Hull-less parent, ZZWW. According to Ubisch (*8*) there is a third factor D concerned in the development of side florets and which is recessive (dd) in the *deficiens* type. *Nudimortoni* must carry the dominant D and the two-row Hull-less dd. The 3 : 13 ratio suggests this two-factor difference. The cross would then be ZZWWdd × ZZwwDD. The F_1 would be ZZWwDd. The genetic classes, observed and expected, in the F_2 generation are given in Table VIII.

TABLE VIII.—*Genetic classes expected and the distribution of 727 F_2 plants obtained from a cross of two-row Hull-less × nudimortoni*

Genotype	Per 16	Observed	Expected	Appearance of the phenotype
ZZDDww 1 ZZDDWw 2	3	154	136.3	Enlarged side florets, all sterile or partially fertile.
ZZDdWw 4	4	160	181.8	Side florets intermediate in size, all sterile.
ZZDDWW 1 ZZDdww 2 ZZDdWW 2	5	390	227.2	Side florets much reduced in size.
ZZddWW 1 ZZddWw 2 ZZddww 1	4	23	181.7	*Deficiens*, side florets absent.
Total		727	727.0	

All classes are homozygous for the Z factor. The individuals carrying the homozygous dominant DD factors are intermediates (F_2 groups 1 and 2 in Table VII) with enlarged side florets all sterile or partially fertile like the female parent, unless the homozygous dominant WW factor is present, in which case the size of the side florets is much reduced. Group 3 apparently contains the plants carrying the heterozygous DdWw factors. The homozygous dominant factors DD and WW reduce the size of the side florets materially. Heterozygous Dd with or without W has a similar effect. The homozygous dd factor results in the absence of side florets. Possibly the presence or absence of W may slightly affect the condition of the side florets.

F_3 Generation

Six hundred and twenty-two F_3 families were grown from the F_2 plants. The F_3 plants were classed in the following classes: (1) Enlarged side florets with one or more fertile, (2) enlarged side florets with none fertile, (3) side florets reduced in size, and (4) side florets absent or practically so (*deficiens* type). The line of demarcation was fairly definite between classes 1 and 2 and between 2 and 3. It was often difficult to separate classes 3 and 4, because many of the families were segregating for spike density. It will be noted that the F_3 classification contains only four classes, whereas the F_2 contained five. In the F_3 families two classes of reduced side florets could not be distinguished. The F_3 results are summarized in Table IX.

TABLE IX.—*Distribution of F_3 families from 622 F_2 plants according to the F_2 classes in a cross of two-row Hull-less × nudimortoni*

No.	F_2 genotypic group description	F_2 group number [1]					Total
		1	2	3	4	5	
1	Plants which gave families containing enlarged side florets, all sterile or partially fertile	37	33	2	1	.....	73
2	Plants which gave families containing enlarged side florets, all sterile	8	42	2	.....	.....	52
3	Plants which gave families containing both sterile enlarged side florets and reduced side florets	.....	11	58	82	2	153
4	Plants which gave families containing both reduced side florets and *deficiens*	.....	.....	7	56	8	71
5	Plants which gave families containing only reduced side florets	.....	.....	8	84	1	93
6	Plants which gave families containing only *deficiens* types	.....	.....	.....	1	1	2
7	Plants which gave families containing enlarged side florets (sterile and partially fertile), and reduced side florets	.....	3	26	38	.....	67
8	Plants which gave families containing enlarged side florets (all sterile), reduced side florets, and *deficiens*	.....	.....	22	50	2	74
9	Plants which gave families containing all classes	.....	.....	10	27	.....	37
	Total	45	89	135	339	14	622

[1] The F_2 groups are as follows:
1. Enlarged side florets, one or more fertile.
2. Enlarged side florets, none fertile.
3. Side florets intermediate in size, all sterile.
4. Side florets much reduced.
5. *Deficiens* type.

Forty-five families were obtained from the F_2 plants in Group 1. These contained only plants with enlarged side florets, most of which had some side florets fertile. From the plants of Group 2, 89 families were obtained. Of these, 75 contained only plants with enlarged side florets, as were obtained from the F_2 plants of Group 1. The

other 14 families each contained some F_3 plants with reduced side florets. It is probable that these 14 F_2 plants belong to Group 3. According to the factorial assumption two-thirds of the F_2 plants with enlarged side florets (Groups 1 and 2) should have produced segregating families, throwing some plants with reduced side florets. Only 14 such were obtained.

From the F_2 plants of Group 3 there were obtained 135 F_3 families. Most of the F_2 plants of this group were heterozygous, giving segregates covering the entire range of F_3 classes, as was expected. The numerical relationships are of little significance. A few apparently homozygous were probably wrongly classified in the F_2.

The plants of the F_2 Groups 4 and 5 produced 339 and 14 F_3 families, respectively. Here, again, the numerical relationships are very indefinite and of little significance. One family contained only plants with enlarged side florets, and the F_2 parent plant probably should have been classed with Group 2. Eighty-five families contained only plants with reduced side florets and 2 contained all *deficiens* plants. The theoretical expectancy is 39 homozygous for reduced side florets and 156 which should give all *deficiens*. There were 201 families which segregated giving some plants with enlarged side florets, while 38 segregated giving some *deficiens*. The theoretical expectancy is 78 in each case.

The expected genotypes and the distribution of the 622 F_2 plants according to the nature of the F_3 progenies produced are shown in Table X.

TABLE X.—*Genetic classes expected and the distribution of 622 F_2 plants, as shown by their F_3 progenies in a cross of two-row Hull-less × nudimortoni*

Genotype	Per 16	Observed	Expected	F_3 segregation
ZZDDww 1	1	125	38.9	Enlarged side florets, all sterile or one or more fertile.
ZZDDWw 2	2	220	77.7	Enlarged and reduced side florets, 1:3.
ZZDdWw 4	4	37	155.5	All four F_3 classes.
ZZDDWW 1	1	93	38.9	All reduced side florets.
ZZDdww 2	2	74	77.7	Enlarged side florets, reduced side florets, and *deficiens*, 1:2:1.
ZZDdWW 2	2	71	77.7	Reduced side florets and *deficiens*, 3:1.
ZZddWW 1 ZZddWw 2 ZZddww 1	4	2	155.5	All *deficiens*.
Total		622	621.9	

DISCUSSION OF RESULTS

Two distinct groups of segregates were produced in the F_2: (1) Those which had enlarged side florets and (2) those which did not. The numbers in these groups were in the proportion of 3 : 13, which suggests the two-factor hypothesis described above. As was suggested in the discussion of the Manchuria × *tridax* cross, the *deficiens* type might be either ZZdd or zzdd. Since no six-rowed plants were produced in F_2, the two-row Hull-less (*deficiens*) must be ZZdd. The two parents differ also in the W factor as indicated by the 3 : 13 ratio. The factorial composition of the parents is as follows: Two-row Hull-less = ZZWWdd, and *nudimortoni* = ZZwwDD.

The agreement between the observed and the calculated F_2 classes (Table VIII) is rather poor. Some of the F_2 plants classed in Group

2 should have been placed in Group 3, as shown by the segregation of their F_3 progenies. With these F_2 plants included in Group 3 the agreement in these classes is much closer. It was also difficult to distinguish between the parental *deficiens* type and those with the size of the side florets much reduced on account of the segregation for spike density. It is possible that the presence or absence of the W factor may have affected the classification of these plants. These two groups combined are probably represented by the last six genotypic classes.

The F_3 results are very unsatisfactory as a verification of the factorial hypothesis. In all classes only two large deviations occur, which can hardly be accounted for through errors in classification. Either the factorial assumptions are incorrect or the development of the side florets in the *nudimortoni* type of intermediate is so affected by environmental conditions as to make the classifications unreliable. No other hypothesis was suggested by these data.

OTHER CROSSES

In addition to the crosses discussed above, the F_2 data are available from 35 other crosses between:

(1) Six-rowed and two-rowed forms,
(2) Six-rowed and *deficiens* forms,
(3) Intermediate and *deficiens* forms, and,
(4) Intermediate and two-rowed forms.

All the parental forms are from pure-line material. In some cases the F_2 progenies are too small to be significant. With the exception of cross 240, none of the crosses have been tested in the F_3 generation.

SIX-ROWED × TWO-ROWED

The F_2 data from 12 crosses between six and two rowed forms are given in Table XI. In these crosses four six-rowed sorts (Featherston, Oderbrucker, Peruvian, and *aethiops*) were used. The two-rowed parents used (McEwans Hull-less, Frankish, New Zealand, *primus*, and Red Barley) are all awned and of the *Hordeum distichon* type, with sterile side florets.

In no case was there complete dominance of the two-rowed character. The side florets of the F_1 plants were intermediate in size, and in some cases they were partially fertile and bore short awns.

TABLE XI.—*Segregation of the F_2 progenies from 12 crosses between two-rowed and six-rowed forms*

Cross No.	Cross	Number F_2 plants	Observed		Calculated 3 : 1		Deviation
			Not 6-rowed	6-rowed	Not 6-rowed	6-rowed	
196	Featherston × McEwans Hull-less.	32	21	11	24.0	8.0	3.0±1.65
199	Frankish × Featherston	54	44	10	40.5	13.5	3.5±2.15
218	Oderbrucker × New Zealand	125	90	35	93.8	31.2	3.8±3.25
219							
243	Peruvian × McEwans Hull-less	120	78	42	90.0	30.0	12.0±3.20
244	Oderbrucker × Frankish	239	163	76	179.3	59.7	16.3±4.52
245							
267	Aethiops × McEwans Hull-less	46	30	16	34.5	11.5	4.5±1.98
268							
272	Red Barley × *aethiops*	20	16	4	15.0	5.0	1.0±1.31
271	Aethiops × Primus	155	125	30	116.3	38.7	8.7±3.64
278							

In general, the six-rowed segregates and those "not six-rowed" occurred in the proportion of 1:3. In the crosses of Peruvian × McEwans Hull-less and Oderbrucker × Frankish, where the deviations are rather large, the number of six-rowed forms are too high, precluding all possiblity of a 13:3 ratio, and the fit to a 9:7 ratio is equally poor. Both progenies are probably 3:1 segregations. These results agree with those obtained from the cross of Odebrucker × *palmella hypianthinum* and with previous work where six-rowed forms have been found to constitute 25 per cent of the F_2 progeny.

Considerable variation was evident among the segregates classed as "not six-rowed" (i. e., those which showed a lower degree of fertility of the side florets than the fully fertile six-rowed type), ranging from forms with highly fertile side florets bearing short awns to the sterile, awnless side florets of the two-rowed forms. The number of phenotypic classes which could be distinguished in the F_2 of the various crosses ranged from 3 to 6. The occurence of these several F_2 classes, which are not shown in Table XI, indicate that the factorial constitution of the parental forms, as in the cross of Odebrucker × *palmella hypianthinum*, is not as simple as would lead to a 1:2 : 1 F_2 segregation, and that at least two factor pairs are concerned.

Six-Rowed × Deficiens

Table XII contains the F_2 results from seven crosses between six-rowed and *deficiens* forms. The six-rowed sorts used were Featherston, *aethiops*, White Hull-less, and Himalaya; while those of the *deficiens* type were *tridax*, *deficiens*, and two-row Hull-less.

In the F_1 from crosses 31 and 73 the side florets were intermediate in size between the parental forms, and were also sterile. In the case of the other crosses the F_1 side florets were very slightly developed, similar to the cross of Manchuria × *tridax*.

In all crosses the F_2 plants showed a 1:3 segregation between six-rowed forms and those "not six-rowed." This agrees with the results from the cross of Manchuria × *tridax* (six-rowed × *deficiens*) where the segregation for six-rowed, intermediate two-rowed forms, and *deficiens* was approximately 1 : 2 : 1. With the exception of crosses 31 and 73, the F_2 segregates could be grouped into three classes: (1) Six-rowed, (2) side florets very slightly developed (heterozygous class), and (3) *deficiens* type. The probability is that the test of the F_2 will show a 1 : 2 : 1 segregation, indicating a one-factor pair difference between the six-rowed and *deficiens* forms. In crosses 31 and 73 the classification of the F_2 segregates was not so simple. In cross 31 four phenotypic classes could be distinguished: (1) Six-rowed, (2) size of side florets reduced, (3) side florets intermediate like the F_1 (smaller than in class 2), and (4) side florets much reduced (nearly *deficiens*). In cross 73 six phenotypic classes were distinguished, varying from fully fertile six-rowed to *deficiens*. The nature of the F_2 progenies for these two crosses indicates that the parental forms differ by more than one factor pair with the production of more than one class of six-rowed forms which are alike phenotypically but differ as to genotype. The F_3 must be grown to prove the presence of more than one factor.

TABLE XII.—*Segregation of the F_2 progenies from 7 crosses between six-rowed and deficiens forms*

Cross No.	Cross	Number F_2 plants	Observed		Calculated 1:3		Deviation
			6-rowed	Not 6-rowed	6-rowed	Not 6-rowed	
111	Tridax × Featherston	96	28	68	24.0	72.0	4.0±2.86
194	Featherston × *tridax*	90	27	63	22.5	67.5	4.5±2.77
195							
152	Deficiens × *aethiops*	157	41	116	39.2	117.8	1.8±3.66
153							
31	Two-row Hull-less × white Hull-less	31	9	22	7.7	23.3	1.3±1.63
73	Two-row Hull-less × Himalaya	42	10	32	10.5	31.5	.5±1.89

INTERMEDIATE × DEFICIENS

Ten crosses were made between intermediate and *deficiens* forms. The intermediate forms used were *nudimortoni*, *nudihaxtoni*, and *cornutum*. *Nudimortoni* has been previously [4] described. *Nudihaxtoni* resembles the six-rowed type, except that the fertile side florets are awnless and the kernels slightly undersize while the central florets are awned. *Cornatum*, like *nudihaxtoni*, is practically a six-rowed form in which the central florets are hooded and the lateral florets awnless. *Nudihaxtoni* and *cornutum* are classed by Harlan (*4*) as intermediates.

In all crosses the side florets of the F_1 plants were but slightly developed, showing a tendency toward dominance of the *deficiens* character.

The segregation of the F_2 generation is shown in Table XIII. The segregates were grouped in two classes as follows: (1) Those with reduced side florets (two-rowed and *deficiens*) and (2) those having enlarged side florets like the intermediate parents (partially fertile side florets in crosses involving *nudimortoni*, and fully fertile, practically six-rowed, in crosses involving *nudihaxtoni* and *cornutum*). In all crosses not involving *cornutum* as the intermediate parent the proportion of the segregates with reduced side florets to those with enlarged side florets was approximately 13 : 3, the deviation in every case being less than three times the probable error. Here the intermediate parent behaves as did the intermediate parent in crosses of *nudimortoni* × two-row Hull-less. For the crosses involving *cornutum* as the "so called" intermediate parent a 3 : 1 segregation for reduced and enlarged side florets was obtained. In the case of cross 47 the deviation was high, more than three times the probable error. However, the excess of plants with fertile side florets precludes any possibility of a 13 : 3 ratio, and the ratio is probably 3 : 1. The *cornutum* parent used in these crosses behaves much the same as the six-rowed parent in the crosses between six-rowed and *deficiens*. In some crosses, where only two or three phenotypic classes are obtained, there is a single genetic factor difference between the parents, while in other crosses, where several classes of reduced side florets are obtained, there are two or more factors concerned. Twenty-five per cent of the progeny are like *cornutum*, as would be

[4] See page 11.

expected if it were a six-rowed form differing from the ordinary six-rowed only in the matter of awns or hoods carried on the side florets. This assumption is further strengthened by the results of a cross between *cornutum* and Himalaya (six-rowed). Twenty-two plants were produced in the F_2, all of which were six-rowed.

TABLE XIII.—*Segregation of the F_2 progenies from 10 crosses between intermediate and deficiens forms*

Cross No.	Cross	Number of F_2 plants	Observed		Calculated		Deviation
			Reduced side florets	Enlarged side florets (like intermedium parent)	Reduced side florets	Enlarged side florets (like intermedium parent)	
8	*Nudideficiens* × *nudimortoni*	98	81	17	(13:3) 79.6	18.4	1.4±2.61
12	*Nudimortoni* × *nudideficiens*	58	42	16	(13:3) 47.1	10.9	5.1±2.00
214	*Deficiens* × *nudimortoni*	12	10	2	(13:3) 9.8	2.2	.2± .91
99	*Nudihaxtoni* × *deficiens*	57	44	13	(13:3) 46.3	10.7	2.3±1.99
295, 297, 300	*Deficiens* × *cornutum*	281	204	77	(3:1) 210.8	70.2	6.8±4.90
47	*Cornutum* × two-row Hull-less	128	84	44	(3:1) 96.0	32.0	12.0±3.30
75, 78	*Cornutum* × *steudelii*	35	24	11	(3:1) 26.3	8.7	2.3±1.73

Crosses 8, 12, and 214, involving *nudimortoni* as the intermediate parent, gave 5 phenotypic classes in F_2 like the crosses of *nudimortoni* × two-row Hull-less, while cross 99 where *nudihaxtoni* was used gave only 3 such classes. This would suggest that *nudihaxtoni* has a different factorial composition than has *nudimortoni*, and this is further suggested by the difference in appearance of the two forms. The side florets of *nudihaxtoni* are completely fertile, while those of *nudimortoni* are only slightly fertile. Crosses 295, 297, and 300 showed only 2, and cross 99 only 3, distinct phenotypic classes, which indicates that the factorial relationship in these crosses is probably more simple than in cross 47, which gave 6 such classes. The two-row Hull-less type apparently has a different factorial composition than the *deficiens* variety used in the crosses between *deficiens* and *cornutum*. Completeness of the F_3 progenies is necessary for drawing any definite conclusions from these crosses with respect to the factorial constitution of the parental forms.

INTERMEDIATE × TWO-ROWED

In Table XIV are shown the F_2 data from six crosses between intermediate and two-rowed forms. The intermediate forms used were: *Nudimortoni, haxtoni intermedium, nudihaxtoni nuditonsum,* and *mortoni nigritonsum*. *Nudimortoni* and *nudihaxtoni* have been previously described.[5] *Nudihaxtoni nuditonsum* and *mortoni nigritonsum* are types in which the side florets are all fertile, of fair size, and awn-

[5] See page 11.

less (glumes with blunt ends), and the central florets have short awns. *Haxtoni intermedium* shows a lower degree of fertility of the side florets than either the *nudihaxtoni nuditonsum* or *mortoni nigritonsum* sorts. The central florets are fully awned, while the side florets are usually awnless. The two-rowed forms used were: McEwans Hull-less, Frankish, and *subcornutum*. McEwans Hull-less and Frankish have been described.[6] *Subcornutum* is a two-rowed, hooded type, with the kernels white and hulled. The hoods on the central florets are slightly elongated.

In all crosses the F_1 was intermediate between the parental forms with respect to size of side florets. The F_1 plants of crosses 202 and 318 produced side florets which were slightly fertile, while those of the F_1 plants of crosses 240, 311, 306, and 308 were all sterile.

The F_2 segregates were divided into two classes: (1) Those with reduced side florets and (2) those with enlarged side florets like the intermediate parent. Crosses 306, 311, and 318 segregated for spring and winter habit of growth. The winter types did not head in the greenhouse. The data, therefore, include only the spring types.

TABLE XIV.—*Segregation of the F_2 progenies from 6 crosses between intermediate and two-rowed forms*

Cross No.	Cross	Number F_2 plants	Observed		Calculated		Deviation
			Reduced side florets	Enlarged side florets (like intermedium parent)	Reduced side florets	Enlarged side florets (like intermedium parent)	
240	*Nudimortoni* × McEwans hull-less	337	199	138	189.6 (9:7)	147.4	9.4±6.14
202	Frankish × *nudihaxtoni*	34	21	13	19.1 (9:7)	14.9	1.9±1.95
311	*Nudihaxtoni nuditonsum* × Frankish	97	63	34	54.6 (9:7)	42.4	8.4±3.30
			74	23	72.8 (3:1)	24.2	1.2±2.88
306	*Mortoni nigritonsum* × McEwans Hull-less	79	58	21	59.3 (3:1)	19.7	1.3±2.60
308	*Haxtoni intermedium* × McEwans Hull-less	204	154	50	153.0 (3:1)	51.0	1.0±4.17
318	*Mortoni nigritonsum* × *subcornutum*	55	42	13	41.3 (3:1)	13.7	.7±2.17

Crosses 240 and 202 show a 9:7 F_2 ratio for reduced and enlarged side florets. However, as a result of growing a part of the F_3 of cross 240, the indications are that for this cross the ratio is 3:1. One hundred and thirty-seven F_3 progenies from as many F_2 plants gave a ratio of 111 homozygous and heterozygous for two-rowed to 26 homozygous for enlarged side florets (*nudimortoni* type). The progenies of the remaining crosses apparently represent a 3:1 segregation (the segregation ratio from cross 311 might be 9:7). No definite conclusions can be drawn from the F_2 segregations obtained from these crosses without the F_3 progenies. The fact that both 9:7 and 3:1 ratios are obtained might indicate that the intermediate forms are not all alike genetically. This is strongly sug-

[6] See page 16.

gested in the difference in appearance of such types as *nudihaxtoni*, where the side florets are all fertile and short-awned, and the *nudimortoni* type in which only a few of the side florets are fertile and all awnless.

Discussion of Results

All the above crosses, in which a six-rowed form was used as one of the parents, produced F_2 progenies in which 25 per cent of the plants were six-rowed (1 six-rowed: 3 "not six-rowed"). These results indicate that there is one essential factor which differentiates six-rowed forms with fully fertile side florets from those two-rowed forms with sterile or staminate side florets, and also a single factor which differentiates the six-rowed forms from the two-rowed *deficiens* forms.

That there is still another factor concerned in addition to the essential factor is shown by the nature of the classes segregating as "not six-rowed." On the basis of a single-factor difference, it would be expected that one-third of the segregates which are "not six-rowed" would be like the other parent (two-rowed or *deficiens*), and that the other two-thirds would be more or less uniform and probably intermediate between the parental forms, like the F_1. That such was not the case for most of the crosses was pointed out in the discussion of the data shown in Tables XI and XII. Crosses of Featherston × *tridax* and *deficiens* × *aethiops* produced progenies which could easily be classed into three classes, the two parental types and an intermediate class. Here the difference is probably a single pair of allelomorphic factors, recessive in the six-rowed and dominant in the two-rowed and *deficiens* forms. For most of the crosses, however, the heterozygous F_2 segregates showed various degrees of fertility of the side florets. Here a second factor is undoubtedly present. Its relation can not be definitely determined without the F_3 data.

The relation between the intermediates, two-rowed, and *deficiens* forms is not clarified by these F_2 data. The progenies from crosses involving intermediate and *deficiens* forms consistently give 13 : 3 ratios for reduced and enlarged side florets, indicating a two-factor difference. This classification is too general, however, to establish any factorial relationship. Crosses involving *cornutum*, on the other hand, give 3 : 1 ratios for two-rowed and intermediate forms. *Cornutum*, although classed as an intermediate, is, on the basis of fertility of the side florets, phenotypically a six-rowed form. The indications are that it has the same genetic composition as the six-rowed forms, and might be expected to behave the same as Featherston or *aethiops* when crossed with *deficiens*. The relation of the factors in intermediate and two-rowed forms is rather indefinite, as is suggested by Table XIV. It is probable that the intermediates differ in their genetical constitution, a suggestion greatly strengthened by a variation in phenotypic appearance.

GENERAL DISCUSSION AND CONCLUSIONS

Practically all the crosses investigated showed that where a six-rowed form was used as one of the parents, 25 per cent of the F_2 progeny were six-rowed, and that these always bred true or were homozygous for the six-rowed character. Nearly all investigators who have made crosses between six and two rowed forms have found this same simple relationship, which has led some to conclude that

there is but one factor differentiating the two types. That there is one essential factor is probably true. The assumption of two factors where the second is a modifier of the essential factor does not vitiate the single-factor relationship if the modifying factor is allowed to affect only the dominant factor concerned in the fertility of the side florets. In the F_2 progeny, 4 out of 16, or 25 per cent, should be six-rowed, having the factorial combinations, zzWW, zzWw, and zzww. These can not be distinguished from each other and can produce only six-rowed forms, and would therefore appear to be homozygous.

As many of the crosses show, there may be a great variation in the segregation of the F_2 progenies with respect to the segregates in which the fertility is less than the fully fertile six-rowed form. Some F_2 progenies may give simple 1 : 2 : 1 segregations in which there is very little difference between the heterozygous and homozygous two-rowed forms. Such was the result in most cases where the cross was between six-rowed and *deficiens* forms. In other crosses, particularly in those between six-rowed and two-rowed, the F_2 was more complex. The results of the cross Oderbrucker × *palmella hypianthinum* can hardly be explained on a simple, one-factor basis. Various degrees of fertility ranging from highly fertile side florets with short awns to two-rowed forms are accounted for by the presence or absence of a modifying factor, W, where the essential factor, Z, for sterility is present. The assumption of two-factor pairs requires that the ZZww class should breed true for the intermediate character. The presence of such a class was not indicated, as shown by the F_3 data in Table VII, although seven such plants were expected. The absence of this class is probably due to the lack of sufficiently large numbers.

From the above-mentioned crosses it is to be concluded that there are two genetic factors, Z and W, differentiating six-rowed from two-rowed and *deficiens* forms. Although in the proportion of six-rowed to those not "six-rowed" a 1 : 3 relationship is obtained, this is to be expected on the factorial hypothesis assumed. Other crosses of which only the F_2 data are available bear out these conclusions. In practically all cases 25 per cent of the F_2 progeny are six-rowed. In some of these crosses, particularly where the *deficiens* form is used, the ratio is a simple 1 : 2 : 1, and in others it is more complex. In all cases where various degrees of fertility are obtained among the segregates "not six-rowed" it is assumed that the F_1 is heterozygous for both factors. On the other hand, a simple monohybrid relationship is possible if the zzWW form of six-row is used instead of the zzww form in crosses with two-rowed forms, ZZWW.

The above results are similar to those obtained by Harlan and Hayes (*5*) and by Ubisch (*7*). The latter found that when six and two rowed forms, both of which are awned, are crossed, the ratio is 6 two-rowed : 6 intermediates : 4 six-rowed; while where awned and hooded sorts were crossed the ratio is 18 two-rowed : 30 intermediates : 16 six-rowed. Either ratio might be mistaken for a 1 : 2 : 1 ratio, which has been found by all previous investigators who have assumed that a single-factor pair is the basis of the F_2 segregation. He assumed two factors Z and W, for lack of fertility (two-rowed), the six-rowed forms being zzWW and zzww. Harlan and Hayes (*5*), like Ubisch, assume two factors, A and B, with this difference, that

the six-rowed forms are represented by the dominant factors AABB and AAbb.

The relation between the *deficiens*, intermediate, and two-rowed forms is somewhat harder to determine. The F_3 data are available for one cross only (two-row Hull-less × *nudimortoni*), and they are not very conclusive. In the above discussion *deficiens* is assumed to have a factorial composition similar to the two-rowed form (*Hordeum distichon*), i. e., ZZWW, while the intermediate form is ZZww. In all crosses between the intermediates (*nudimortoni* and *nudihaxtoni*) and *deficiens*, the F_2 ratio was 3 with enlarged side florets to 13 with reduced side florets, a two-factor ratio. There must, therefore, be a third factor, D (suggested by Ubisch (*8*)), which is D in the intermediate and d in the *deficiens* forms. *Deficiens* is, therefore, ZZWWdd, and the F_1 (Z) WwDd. From the segregation in the F_3 it is concluded that the enlarged side floret plants are (Z) wwDD and (Z) WwDD. On the other hand, *deficiens* and six-rowed forms differ by a single factor and in the F_2 segregate into 3 *deficiens* : 1 six-rowed. This is entirely possible, since without the factor Z for two-row it can not be determined whether D is present or absent in the six-rowed forms. ZZWW(d) (*deficiens*) × zzWW(d) (six-rowed) would give a 3 : 1 ratio for *deficiens* and six-rowed. More data must be obtained from crosses involving *deficiens* and intermediate, and *deficiens* and two-rowed forms, to determine the relation of the *deficiens* factor.

SUMMARY

The following crosses were carried through the F_2 and F_3 generations:

Manchuria (six-rowed) × *tridax* (*deficiens*).
Oderbrucker (six-rowed) × *palmella hypianthinum* (2-rowed).
Two-row Hull-less (*deficiens*) × *nudimortoni* (intermediate).

Certain conclusions may be drawn from the data obtained:

(1) In some crosses lack of fertility of the side florets is a partially dominant character.

(2) In both crosses where the six-rowed form was used as one of the parents, 25 per cent of the F_2 progeny were six-rowed. It is concluded that there is one essential factor, Z, which differentiates the six and two rowed condition of fertility. In the cross of six-rowed by *deficiens* no other factor was concerned, or, if a second factor was present, it was the same in both parents.

(3) A second factor, W, when present with the Z factor, produces various degrees of fertility of the side florets. Its presence or absence has no effect on the phenotypic appearance of the six-rowed forms, so that the six-rowed forms appear to be homozygous, although the factor W may or may not be present.

(4) The *deficiens* character is due to a third factor, D, for fertility of the side florets. The *deficiens* form carries the recessive d. The D factor is also assumed to be recessive in the six-rowed forms; its expression as absence of the side florets can not occur without the Z factor.

The above conclusions are supported by the F_2 data of a number of other crosses involving the following forms:

Six-rowed × *deficiens*.
Six-rowed × two-rowed.
Intermediate × *deficiens*.
Intermediates × two-rowed.

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A LEAF, BRACT, AND BOLL SPOT OF SEA-ISLAND COTTON CAUSED BY HELMINTHOSPORIUM GOSSYPII N. SP. [1]

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INTRODUCTION

The only variety of cotton grown on a commercial scale in Porto Rico is sea-island (*Gossypium barbadense* Linn.), and it is cultivated principally in the southwest section of the island, in the vicinity of Lajas, and along the northwest coast, near Quebradillas, Camuy, and Hatillo, sections which are too dry for sugar cane. During the last three seasons specimens of diseased leaves, bracts, and bolls have been received from both sections. The disease was investigated and the results of the investigation are given here.

THE DISEASE

The leaves, flower bracts, and bolls are attacked. The spots on the leaves and bracts are 1 to 8 mm. in diameter and often so numerous as to be confluent. When infection first occurs the spots are light red, gradually darkening to dark purple. With age, the centers become brown and finally ashen. In old spots the centers often break and fall away, leaving jagged holes in the leaf (fig. 1). The bolls are infected when young, but the hardening of the tissues prevents the growth of the fungus from forming large spots, and the damage to the bolls is confined to small dotlike purplish lesions, which so far have not been observed to penetrate to the fiber and seeds. The damage caused by the disease is therefore probably confined to the destruction of leaf tissue and the early defoliation which ensues when infection is severe. Infection is general in the fields in the southwest section of the island, and only very young leaves are free from infection.

THE ORGANISM

Numerous examinations of diseased material showed the constant presence of the conidiophores and conidia of a Helminthosporium on both sides of the ashen centers of old spots. The fungus was isolated repeatedly from spots on the leaves, bracts, and bolls.

The dimensions of the conidia are quite constant, whether produced under moist or dry conditions. The extremes noted among 400 conidia measured were 35 to 118 × 11.7 to 18.4 μ. These figures indicate great variation, but a large majority of the spores fall within much more restricted limits, 85 per cent of them occurring within the length limits of 70 to 115 μ, while 95 per cent are from 13 to 17 μ in diameter. The average dimensions were 87 × 15.3 μ.

[1] Received for publication Aug. 4, 1925; issued February, 1926.

[2] The writer is indebted to C. L. Shear, of the Bureau of Plant Industry, United States Department of Agriculture, for examining the literature for references to a Helminthosporium on cotton.

The conidia are 1 to 8 septate, 85 per cent having from 4 to 7 septa. They are smooth, rounded at the ends, elliptical, usually curved but occasionally straight. They are light to dark fuliginous in

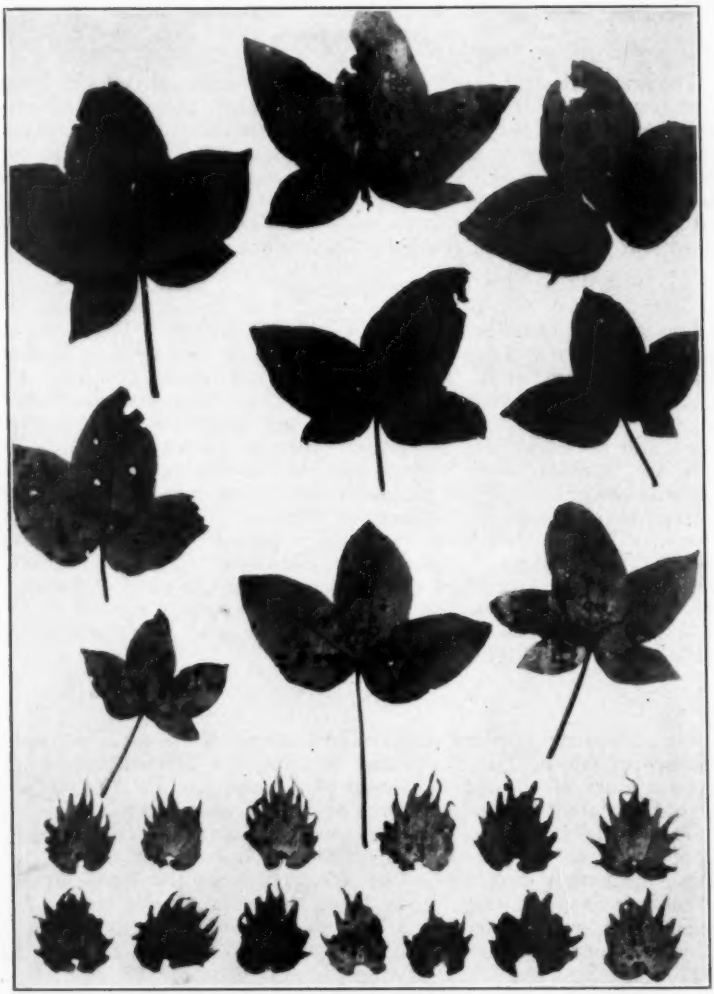

FIG. 1.—Leaves and flower bracts of sea-island cotton attacked by *Helminthosporium gossypii* n. sp. Natural infection

color and densely granular, and become more granular and darker with age. The hilum is visible only under high magnification, and is included in the basal contour (fig. 2).

The conidia germinate quickly (fresh conidia within 30 minutes) by the production of germ tubes. The first tube produced is usually from the apical cell, from which the young tube is pushed through the thin-walled apex. This germ tube is usually followed by one or two tubes from the basal cell. Upon germination a germ tube does not push through the hilum but through the base of the spore adjacent to it. The germination of the apical and basal cells is often followed by the germination of a central cell. Thus, the average spore produces one to four germ tubes.

The conidiophores are amphigenous on the ashen centers of the leaf and bract spots. They are not often found on the boll spots. They arise from stomata, or from between epidermal cells, either singly or in groups of three to six. The conidiophores vary greatly in color, length, and shape. They may be concolorous with the conidia or lighter or darker and apparently become darker with age. Old conidiophores may resume their growth under favorable conditions, and some may be found which are dark brown below, with new apical growth which is almost hyaline and colorless. Those observed were 1 to 5 septate and 40 to 185 × 6.5 to 8.5 μ. The measurements and the number of septa are so variable that they offer no reliable basis for the separation of this from other species.

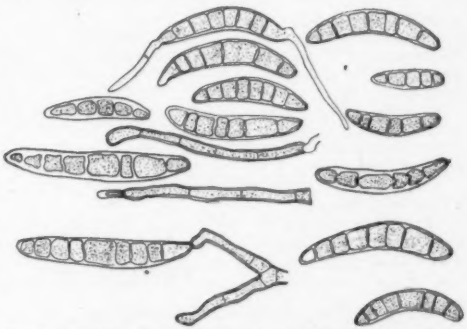

FIG. 2.—Conidia, conidiophores, and germinating conidia of *Helminthosporium gossypii* n. sp., from leaves of sea-island cotton. × 540

Germinating conidiophores have been observed on moist cotton leaves. One to several germ tubes grow from near the apex. They are of about the same diameter and appearance of those of germinating conidia, and doubtless are equally capable of entering the host and producing infection.

The fungus grows well on culture media, and varies greatly in its cultural characters with the medium on which it is planted.

Growth on potato agar: Very thin, appressed, white, spreading. Colonies circular and regular. After five days they have an average diameter of 47 mm. After a week the colonies are still white, and have borne no conidia.

Growth on potato-dextrose agar: Abundant, thick, dense, mostly appressed, dark olive from above, almost black from below, with a narrow white margin. Colonies somewhat irregular in outline. Average diameter of colonies after five days' growth is 26 mm. After a week conidia are present in great numbers; they are slightly shorter, darker, and more granular than those borne on cotton leaves, but in shape, number of septa, and diameter no differences were observed. In older cultures the septa become indistinct, due to the darkening of the cell contents.

Growth on oatmeal agar: Dense, appressed, dark olive, with a narrow white margin. Colonies somewhat irregular in shape, with an average diameter of 33 mm. after five days' growth. After a week there is an abundance of olive mycelium, but no conidia have been borne.

Obviously the fungus finds sugar necessary for color production and spore formation.

No record of the occurrence of the fungus on cotton or closely related plants was found in the literature. Since the species differs markedly, both in nature and in pure culture, from the species previously recorded from Porto Rico, it is described here as a new species under the name *Helminthosporium gossypii*.

Helminthosporium gossypii n. sp.

Attacks the leaves, flower bracts, and bolls of sea-island cotton (*Gossypium barbadense* Linn.), causing on leaves and bracts numerous purplish circular spots 1 to 8 mm. in diameter which become brown or ashen in the centers. On bolls the spots are punctate and purplish, not enlarging or becoming differentiated in color in the centers.

Conidiophores amphigenous, in the brown or ashen centers of spots, arising from stomata or from between epidermal cells singly or in groups of three to six; brown; variable from straight cylindrical to nodose or bent and geniculate, continuous to 5 septate, 40 to 185 × 6.5 to 8.5 μ.

Conidia light to dark fuliginous, thick walled, elliptical, typically somewhat curved but occasionally straight, rounded at the ends; hilum inconspicuous, included within the basal contour; germinating, usually by 1 to 3 polar and 1 central germ tube, 1 to 8 septate, the septa not marked by constrictions, 35 to 118 × 11.7 to 18.4 μ. Average dimensions, 87 × 15.3 μ. About 85 per cent are 4 to 7 septate.

HABITAT.—Collected on living leaves, bracts, and bolls of *Gossypium barbadense* Linn., at Lajas, Quebradillas, and Isabela, Porto Rico, in 1923, 1924, and 1925.

PATHOGENICITY

To demonstrate the parasitism of the fungus, three series of inoculations have been made, at varying seasons of the year, in 1924 and 1925. The age of the inoculated plants varied from one to four months. The inoculations were performed at Mayaguez, where cotton is not grown, and the plants were entirely free from natural infection. The fungus was grown on potato-dextrose agar for two weeks, at the end of which time conidia were abundant. The cultures were then shaken up with sterile water and the suspension was atomized upon all parts of the plants.

Inoculation was followed in every case by the appearance of spots on the leaves, bracts, and bolls identical with those of natural infections. The earliest visible symptoms of infection appeared three days after inoculation. Very faint reddish rings 1 to 2 mm. in diamter appeared on the inoculated leaves and bracts. At this stage the fungus was not visible on the exterior, but upon plating bits of surface-sterilized tissue the Helminthosporium was constantly recovered. After seven to nine days the spots had enlarged, become purple on the margins and brown in the centers, where conidiophores and conidia were being produced. Infection of young bolls proceeded more slowly, and the first evidences of infection appeared on them after six to eight days. These inoculations, thrice repeated on more than 100 plants, seem to leave no doubt as to the pathogenicity of the fungus. Control plants separated from the inoculated plants did not become infected.

TRANSMISSION

The disease is not transmitted through the seed. Observation indicated that the fungus did not attack the fiber and seed, which was confirmed by negative attempts to isolate the organism from seed from infected bolls. Such seed, planted at Mayaguez, produced plants which remained free from infection.

The most important source of infection is the infected plants which remain in the fields. The crop is grown seasonally, and if the closed season now advocated for the control of the pink bollworm (*Pectinophora gossypiella* Saunders) is practiced, it is believed that the Helminthosporium disease will decrease.

INFLUENCE OF MOISTURE ON THE DISEASE

During the winter of 1924 it was noticed that some inoculated plants which had not been watered for some time following inoculation seemed to have more infected leaves than others which were receiving water more frequently.

Parallel series of inoculations were made. One series was watered daily, wetting the entire plant. The other series was watered only when it seemed necessary to keep the plants growing. The initial infection in the two series was about the same, but there was a decided difference in the amount of secondary infection. The plants grown under dry conditions continued to show infection of new leaves, and the plants were soon typical of those in the fields near Lajas, where severe droughts are of frequent occurrence.

The series of plants watered daily showed very little secondary infection, and when the originally infected leaves had fallen the plants were nearly free from Helminthosporium, but they were heavily infected by the areolate mildew fungus (*Ramularia areola* Atk.).

Whether the above results indicate that *Helminthosporium gossypii* infects drought-weakened wilted leaves more than turgid growing leaves, or that the fungus can not compete with *Ramularia areola* under humid conditions, has not been determined. The fact remains that the fungus is capable of doing more injury under drought than under humid conditions.

SUMMARY

A disease of sea-island cotton causing spots on leaves, flower bracts, and bolls is present generally in the principal cotton-growing sections of Porto Rico.

A species of Helminthosporium was isolated from the diseased tissues, studied in pure culture, and the disease was reproduced by the inoculation of unwounded tissues.

The fungus is described as *Helminthosporium gossypii* n. sp.

The fungus does not infect the seeds, and no infected plants were obtained from seed from diseased bolls.

The disease is more severe under drought than under humid conditions.